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Decoupling the Effects of Atmospheric Humidity and Soil Moisture on Cereal Physiology

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ABSTRACT

Climate change is driving perturbations to global humidity levels with rises and falls already observed across many of the crop growing regions whilst drought continues to threaten agricultural productivity. Soil moisture content determines how much water can be supplied to a plant and atmospheric humidity influences vapour pressure deficit (VPD) thus driving transpirational demand. The influences of both this supply and demand on plant physiology are often overlooked, with drought study research seldom focused on the possible influences of humidity. This thesis aims to address this problem by decoupling the effects of humidity and soil moisture on maize (*Zea mays*) and wheat (*Triticum aestivum* cv. Paragon) through controlled growth under four treatments; high humidity high soil moisture, high humidity low soil moisture, low humidity high soil moisture, low humidity low soil moisture. This thesis found distinct differences in response between young maize and wheat crops with maize appearing more sensitive to humidity and wheat more to soil moisture. There was also variation between species in which areas of physiology were influenced most by the treatment conditions, as maize showed more responses related to biomass production and root architecture, whereas wheat gas exchange and stomatal morphology were more heavily impacted. This thesis highlights the importance of studying the effects of humidity and soil moisture concurrently as both maize and wheat were affected by individual treatments as well as significant interactions between the two. There is also evidence that high humidity could be mitigating some effects of low soil moisture conditions in the early growth of both species, which could have big impacts on future irrigation practices, commercial glasshouses, and predictive hydraulic and climatic models.

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THESIS STRUCTURE

Chapter 1 is a summary of the literature related to humidity and soil moisture influences on plant physiology. This chapter will introduce the areas of physiology explored throughout the thesis.

Chapter 2 details an experiment carried out within a growth chamber situated in a glasshouse, investigating the effects of humidity and soil moisture on plant biomass production, stomatal morphology, and photosynthetic capacity.

Chapter 3 continues with the same growth set up as Chapter 2, whilst investigating the effects of humidity and soil moisture on the concentration of ABA in the roots and shoots.

Chapter 4 follows on from previous chapters with the same growth set up. This chapter details and experiment which is looking at how humidity and soil moisture influence root anatomy and leaf cuticle chemistry.

Chapter 5 builds on findings from Chapter 2 whilst carrying out the experiments in controlled growth chamber conditions. This chapter explores areas of interest that were highlighted in Chapter 2 in greater detail. The effects of humidity and soil moisture on plant biomass, stomatal morphology, root system architecture, gas exchange and chlorophyll fluorescence are all explored in this chapter.

Chapter 6 summarises the overall outcome of the thesis whilst looking at the bigger picture and implications for future research.

1 LITERATURE REVIEW

1.1 GENERAL INTRODUCTION

Global climate change coupled with exponential population growth is placing a colossal burden on our natural resources and is affecting all aspects of global food security including production, availability and access (IPCC, 2017). At present, global cereal crop productivity needs to increase by 39%, topping four billion metric tonnes (Shah and Wu, 2019), to support a population predicted to reach 10 billion by 2050 (Truong, McCormick and Mullet, 2017). Increased food production in both field and controlled environment agriculture, requires a deeper understanding of factors affecting plant growth and productivity, to create efficient growth regimes suited to the prevailing environmental conditions, minimising resource inputs (e.g. water and nutrients) and maximising outputs (yield). Some climatic models (Soden *et al.*, 2005; Wentz *et al.*, 2007) predict global warming driven by rising atmospheric CO_2 will intensify existing climatic problems, leading to changes in atmospheric water vapour content and precipitation (volume and regularity) (Perdomo *et al.*, 2017), thus affecting relative humidity and soil moisture conditions, around the globe.

Perturbations in relative humidity levels are already being observed, with increases recorded in central and eastern United States, western China (Dai, 2006), central Europe (Jones and Moberg, 2003), eastern Africa (Collier *et al*, 2008) and falling humidity levels reported in the UK (Met Office, 2012), and South Africa (Collier *et al.*, 2008). Relative humidity is tightly linked to vapour pressure deficit (VPD), which, is the difference between the saturation vapour pressure (100% relative humidity) and the air pressure at a given temperature

(Amitrano *et al.*, 2019). From a plant perspective, the difference in vapour pressure between the internal leaf environment and the surrounding atmosphere is perceived directly by the plant (Wheeler and Stroock, 2008), as such VPD drives transpiration (Shamshiri *et al.*, 2018) which is actively controlled by the stomatal aperture (Sulman *et al.*, 2016) in vascular plants. Under low humidity conditions, the difference between plant and atmosphere is large (so VPD is high), resulting in high evapotranspiration rates (Taiz *et al*, 2014). When transpiration exceeds soil water availability, stomata close to prevent loss of turgor and associated stress reducing carbon gain and overall productivity potential and possibly raising leaf temperature further. In contrast, high humidity leads to low VPD (Shamshiri *et al.*, 2018), reducing evapotranspiration (Burgess and Dawson, 2004). Fog suppression of evapotranspiration through reduced radiation, temperature and VPD was seen to reduce dry-season water deficits by 25% at watershed scales (Chung *et al*, 2017). As such, changes to global relative humidity levels across major crop growing regions (Figure 1) will have a significant impact on VPD and the subsequent transpirational demands placed on plants.

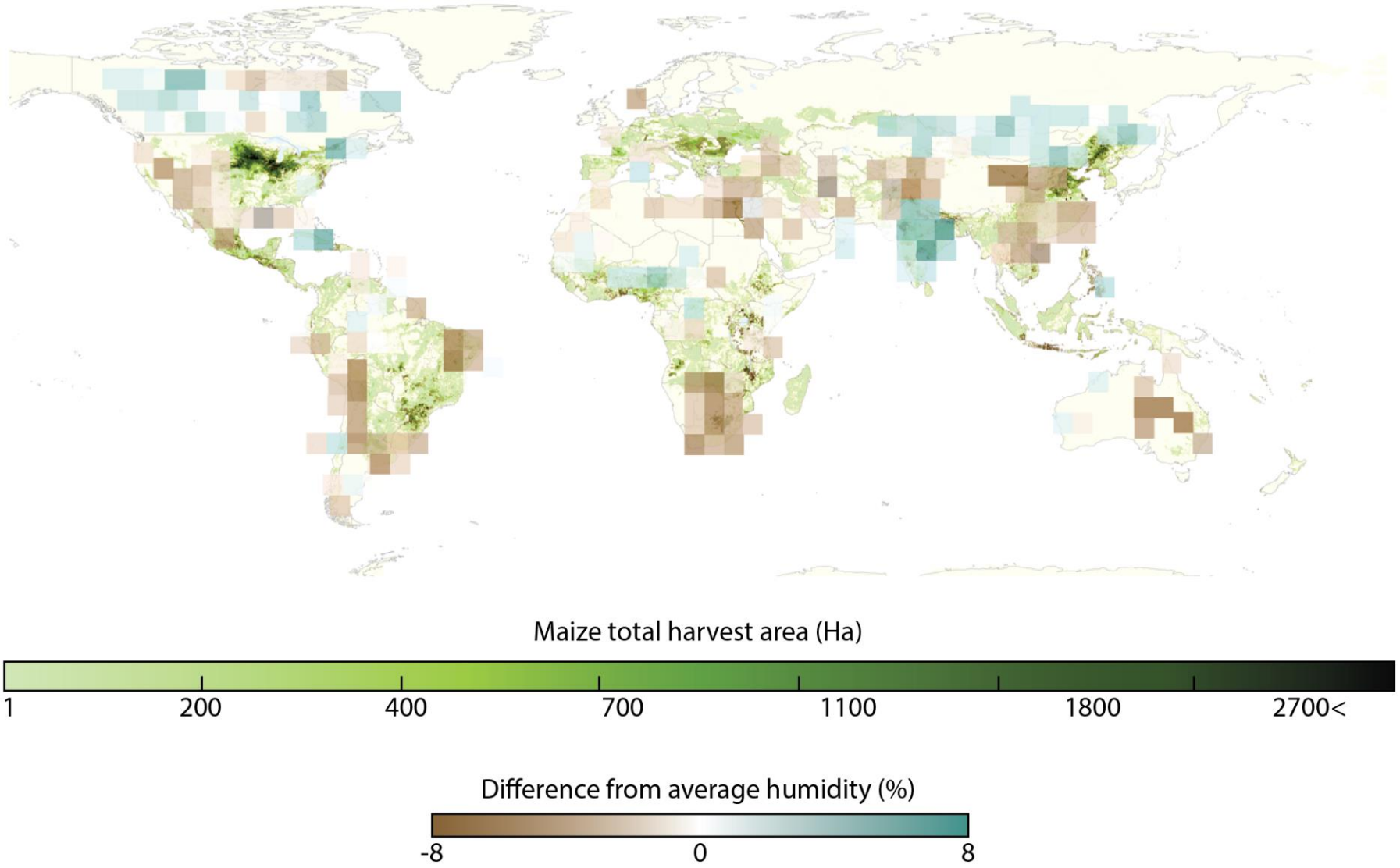


Figure 1. Schematic representing the changes in global humidity levels (both increases and decreases) across areas of major crop production, in this example, Maize. The schematic is a combination of two different global maps. Adapted from Plate 2.1(g) in State of the Climate 2013 (Blunden *et al.*, 2014) showing changes to humidity levels and the maize total global harvest area (Ha) from (You *et al.*, 2014). The difference in humidity is the relative humidity over land in 2013, compared to the 1981-2010 average. Shades of blue indicate where the air was up to 8% more humid than average whereas shades of brown indicate where the air was up to 8% drier (less humid) than average. NOAA map Dan Pisut, based on HadISDH data. The maize harvest is spatially disaggregated production statistics circa 2005 using the Spatial Production Allocation Model (SPAM) for maize harvest predictions 2013. This schematic aims to illustrate the major crop-growing regions around the globe and highlight where humidity changes are occurring, for example, increased humidity over India reduced humidity affective South Africa.

In addition to changing humidity conditions, global soil moisture levels are declining, and recent predictions for Europe show further reductions to soil moisture content irrespective of emission scenario, with a rise in severe soil water droughts both in duration and intensity (Grillakis, 2019). The optimisation of vital resource usage such as water is a challenge for those promoting sustainable agriculture in arid and semi-arid regions (Medrano *et al.*, 2015) and whilst the impacts of drought, temperature and light intensity on plant productivity has been extensively studied on crops, including maize and wheat (Tao *et al.*, 2016; Akter and Rafiqul Islam, 2017; Zhang *et al.*, 2018; Hussain *et al.*, 2019; Nisa *et al.*, 2019), the influences of humidity and subsequently vapour pressure deficit (VPD) are only recently being investigated. The importance of studying both soil moisture and relative humidity is highlighted in a study by Sánchez-Díaz *et al.*, (2002) looking at the effects of soil drought and atmospheric humidity on the yield and gas exchange of Barley. A relatively recent study (Novick *et al.*, 2016) concluded that low humidity (high VPD) to be a bigger driver of plant stress than dry soil conditions, drawing attention to the importance of investigating both humidity (and subsequent VPD) and soil moisture, and raising significant questions regarding the influences of VPD plant water relations in a changing climate.

The control of microclimate factors such as relative humidity could go a long way in managing transpiration and overall VPD pressures on plants in commercially grown controlled environments (Santosh *et al.*, 2017; Amitrano *et al.*, 2019), as well as potentially relieving some of the stress placed on field-grown plants by low soil moisture conditions.

In addition to their importance in global food security, plants, through photosynthesis and transpiration play a pivotal role in carbon and water cycles, bridging Earth system and plant-climate feedbacks (Hetherington and Woodward, 2003; Keenan *et al.*, 2013; Schlesinger and Jasechko, 2014). Only a very small proportion (around 5%) of water passing through the plant, is utilised or stored in plant tissues (Amitrano *et al.*, 2019), the rest is lost to the atmosphere via transpiration. This can represent a significant water flux as plants in the tropics alone transpire 32×10^{15} kg yr^{-1} of water (double the water content of the atmosphere (Hetherington and Woodward, 2003). Factors affecting transpiration rates such as water supply (soil moisture content) and transpirational demand (driven by VPD) humidity (Shamshiri *et al.*, 2018), will have not only a significant effect on whole plant physiology but also global hydrological cycles. Despite the importance, plant responses to atmospheric humidity and subsequent VPD, are not fully understood and increased knowledge is required to improve predictive climatic and vegetative models (Grossiord *et al.*, 2020). Furthermore, there is an intrinsic link between relative humidity and soil moisture content, as climate-induced VPD values greater than 2 kPa (low humidity), have been predicted to exacerbate physiological stress under water deficit conditions, through enhanced plant water loss or reduced carbon gain, depending on soil water availability and inherent plant behaviours (isohydric/anisohydric) (McDowell *et al.*, 2008; Sade, Gebremedhin and Moshelion, 2012; Anderegg *et al.*, 2015; McDowell and Allen, 2015). Given the growing need to increase plant productivity, understand the impacts of climate change on food security and predict future changes to the environment, it is crucial to further our

understanding on plant responses to soil moisture and humidity and investigate whether high humidity and subsequently low VPD can mitigate the negative effects of low soil moisture content on whole plant physiology.

1.2 WATER MOVEMENT

1.2.1 Soil-Plant-Atmosphere Continuum (SPAC)

There exists a traditional view of water movement whereby soil water is taken up by roots, travels up through the xylem before being transpired to the atmosphere, aiding the control of leaf temperatures through evaporative cooling (Ball, Cowan and Farquhar, 1988). This movement of water is commonly referred to as the Soil-Plant-Atmosphere Continuum (SPAC) (Jones, 2013) and is the product of varying water potentials within the plant. When a plant transpires and loses water through stomatal pores, leaf water potentials are consequently lowered establishing a water potential gradient within the plant (Jones, 2013). This gradient from soil (higher water potential) to leaves (lower water potential), pulls water up through the plant via the cohesion-tension theory (Dixon and Joly, 1895) whereby sap flow is maintained by cohesive forces within the water column and the strong adhesion of water to the walls of the xylem cells. It is estimated that these water columns can withstand tensions of up to 10 MPa, enabling water transport to the very top of the tallest trees (Steudle, 2001), overcoming the gravitational forces from the ground below. With soil moisture providing the ‘supply’ of water in this system and transpiration creating the ‘demand’, changes to soil moisture content and humidity (and subsequent VPD) will have a significant impact on water movement throughout the plant system. When demand exceeds supply, there can be significant knock-on effects to the integrity of the water column and subsequent water supply.

1.2.1.1 Cavitation

Regardless of the substantial tensile strength of the water column, when critical tension is achieved in the lumen of xylem vessels (Steudle, 2001), air can be drawn in and fill conduits (embolism), resulting in the breakage of the water column (cavitation) (Steudle, 2001; Jones, 2013). Cavitation and the resulting embolisms are therefore detrimental to the cohesion-tension of water movement throughout the plant. As such, environmental conditions such as low humidity (high VPD) increasing the transpirational demand, if not suitably balanced with soil water supply, could place a plant at higher risk of cavitation and embolism.

Despite the damaging effect of cavitation and embolism, it is a relatively common occurrence in plants, therefore several recovery strategies have been developed. Firstly, when embolism occurs, water can seek an alternative path of least resistance (de Campos Siega *et al.*, 2018), enabling the continuation of water movement throughout the plant. Secondly, the dissolution of entrapped gases can occur when xylem pressure potentials rise, usually driven by reduced transpiration and increased root pressures occurring more commonly at night (Jones, 2013).

1.2.2 SPAC Deviance – Foliar Water Uptake and Hydraulic Redistribution

For the SPAC to function, a gradient of decreasing water potentials between soil root interface and the leaf atmosphere interface needs to be maintained. We are only recently coming to understand that a “bottom-up” approach is not the only mechanism for which water can move throughout a plant. The traditional SPAC can be quite literally turned on its head when root water potential drops below leaf water potential, resulting in a reversal of sap flow (Schreel and Steppe, 2020), this phenomenon only requires leaf water potential to be slightly higher than that of

the root, as the water movement is helped by gravitational water potential, rather than hindered (as seen in SPAC) (Schreel *et al.*, 2019). The reversal in sap flow is responsible for hydraulic redistribution of water towards drier regions of the plant including the roots (Nadezhdina *et al.*, 2010). The apparent backpedalling of plant sap from leaves to roots is predominantly driven by foliar water uptake, providing water directly to foliar sites (Limm *et al.*, 2009) and raising plant water potentials (Breshears *et al*, 2008; Limm *et al*, 2009).

1.2.2.1 Foliar water uptake (FWU)

The exact mechanisms and pathways of foliar water uptake are not fully understood (Burgess and Dawson, 2004). Currently, we know that there are three distinct ways by which water can enter a leaf (1) liquid water from precipitation can be directly absorbed, (2) atmospheric water can condense onto a leaf where it pools as liquid water and then absorbed, (3) water vapour can enter into the sub-stomatal cavity where it then condenses into liquid water and then absorbed (Schreel and Steppe, 2020).

How exactly the water enters the leaf is still hotly debated amongst the literature (Figure 2), with suggestions that the foliar water can diffuse directly through the cuticle (Ketel, Dirkse and Ringoet, 1972; Yates and Hutley, 1995; Kerstiens, 1996; Gouvra and Grammatikopoulos, 2003; Limm *et al.*, 2009), or is absorbed through stomatal pores (Burkhardt, 2010), trichomes (Martin, 1994; Ohrui *et al.*, 2007) or hydathodes (Martin and Von Willert, 2000) (Figure 2). It is possible that multiple entry pathways are possible, which could help to explain the diversity of leaf uptake capacities between species and even within individual plants with older leaves considered to be more efficient at foliar water uptake than younger

leaves (Burgess and Dawson, 2004). Most recently, it has been suggested that stomata could be the key players, with reverse transpiration through stomatal pores accounting for most of the water absorbed by foliar water uptake (Binks *et al.*, 2020).

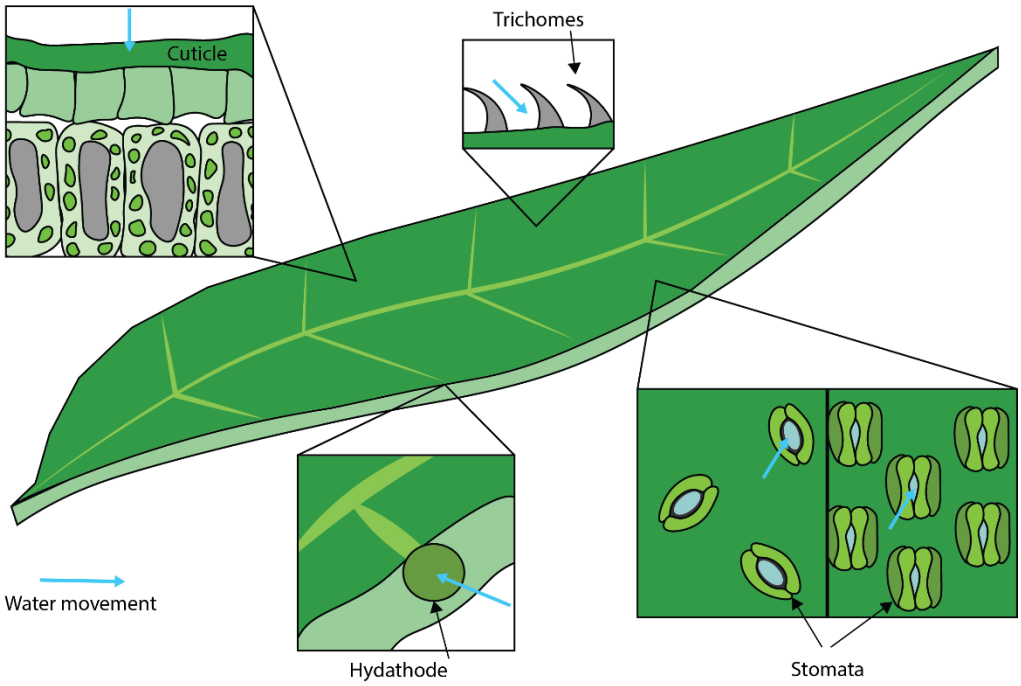


Figure 2. Schematic of the four proposed entry points of foliar absorbed water. Water vapour can enter stomata through a process of reverse transpiration, whereas liquid water can be directly absorbed through the leaf cuticle, hydathodes or trichomes. Adapted from (Schreel and Steppe, 2020).

1.2.2.2 Implications

Historically, the importance of foliar water uptake has often been omitted from water transport research, since up until relatively recently, foliar water uptake was only thought to occur during leaf wetting events (Eller, Lima and Oliveira, 2013; Goldsmith, 2013). We are now beginning to understand that only a water source and favourable water potential conditions are required for foliar water uptake to occur and drive a reversal in sap flow (Eller, Burgess and Oliveira, 2015). A

prime example of the impact foliar water uptake can have on plant survival when Dawson (1998) found fog contributes a striking ~66% of water accessed by understory plants and 19% of the water demand for Californian redwoods. This foliar absorption can cause a reversal of sap flow in the redwoods (Burgess and Dawson, 2004) providing valuable water to dry roots. A study on FWU of artificial dew of Eastern white pine (*Pinus strobus*) seedlings (Boucher, Munson and Bernier, 1995), observed enhanced root growth during high dew treatments, with the effects of dew on root growth larger during low soil moisture treatment compared to well-watered conditions. Hence the importance of studying the effects of soil moisture and humidity on plant water movement. When environmental conditions lead to plants growing in low soil mositure conditions, brought about through drought, growth on steep slopes (Nadezhdina *et al.*, 2010), saline conditions (Steppe *et al.*, 2018), or conditions encountered by shallow rooting species (Tognetti, 2015), low soil water potentials are promoted. Couple these low soil water potentials with increasing humidity (lowering the VPD and transpirational demand), and there may be more instances where foliar water uptake can provide a valuable water subsidy (Breshears *et al*, 2008; Limm *et al*, 2009).

A recent meta-analysis carried out by Schreel and Steppe (2020) showed over 180 species are capable of foliar water uptake, this phenomenon is no longer associated with a few individuals. Also, recent research has suggested that precipitation events leading to leaf wetting occur over 100 days per year in all ecoregions of the world (Dawson and Goldsmith, 2018). Both the number of foliar water uptake competent species and suitable environmental conditions that

promote the strategy suggests that changes to foliar water uptake rates and potential reversal in sap flow could have significant implications to crop production and hydrological cycles worldwide (Goldsmith, Matzke and Dawson, 2013). Leading to the increasing interest in the area, with more research being carried out on the general importance of foliar water uptake (Dawson and Goldsmith, 2018; Berry *et al.*, 2019), as its function under a changing climate remains very much uncertain.

1.2.2.3 Factors affecting foliar water uptake capacity

Foliar water uptake and subsequent reversal of sap flow not only depend on the right environmental conditions albeit a water source and lower root water potentials compared to leaf, but also on the hydrophobicity of the leaf itself. The chemical structure of the leaf structure can change during high humidity conditions, reducing the hydrophobic properties of the leaf, promoting foliar water uptake, but increasing leaf susceptibility to fungal attack (Oksanen *et al.*, 2018). Cuticular properties that influence the boundary layer, affecting the vapour pressure gradient at the leaf-air interface, will affect potential foliar water uptake (Berry *et al.*, 2019). Cuticular wax depositions, stomatal morphology and trichomes can all influence water retention and boundary layer properties (Brewer, Smith and Vogelmann, 1991; Brewer *et al.*, 1997 reviewed in (Rosado and Holder, 2013) These cuticular characteristics can differ considerably between species and even on leaves within the same plant (e.g. affected by leaf age), therefore promoting differential fluxes of foliar water uptake (Berry *et al.*, 2019).

Though these properties and subsequent changes to the boundary layer not only affect the potential for foliar water uptake but also 'traditional' water movement throughout the plant (SPAC), influencing whole plant physiology.

As such, the plant-atmosphere interface will now be discussed, along with subsequent effects on water movement (both SPAC and foliar water uptake), to highlight the importance of considering the influences of these cuticular characteristics at the leaf level and plant level under varying soil moisture and humidity regimes.

1.3 PLANT-ATMOSPHERE INTERFACE

1.3.1 Stomatal Morphology

Stomata are key morphological adaptations when considering a plant's response to humidity and soil moisture. These 'gatekeepers' dictate the passage of gases and water between the internal plant environment and the external atmosphere (Jones, 1998; Brodribb and McAdam, 2011). When open, stomata allow carbon dioxide to enter and be converted to sugars for building plant tissues. However, open stomata also result in water loss via transpiration. If not adequately controlled, transpiration leads to dehydration and death, so stomatal aperture is closely regulated by a series of signals within the plant.

Studies concerning humidity, have found stomata can respond to changes in humidity independently of root signalling (Holbrook *et al*, 2002). For example, stomata in epidermal strips of *Polypodium vulgate* and *Valerianella locusta* which were removed from the leaf (and all associated plant water status influences) closed on exposure to dry air (~20% RH) and opened when exposed to humid air (~70%) (Lange *et al.*, 1971). Although this experiment showed stomata were able

to close independently of root influences, the signal responsible for the closure was not identified. It could also be argued as to how representative such experiments are of whole plant responses, considering the isolated nature of epidermal strip experimentation. Since then, there have been numerous studies showing ABA has a role in stomatal response to changes in humidity in Arabidopsis with increased ABA synthesis occurring under lower humidity conditions (Ikegami *et al.*, 2009; Okamoto *et al.*, 2009; Bauer *et al* 2013). During low soil moisture availability, the signals move from the root to the shoots to close stomata and limit water loss (Rogiers *et al.*, 2012). The signal itself has been debated over the years, the most recent consensus being that plants require both hydraulic and ABA-induced signals. For a comprehensive review on signalling see Buckley (2019).

Regardless of the origin of the signal, stomata need to effectively respond to changes in soil moisture conditions and transpirational demand driven by VPD (and ultimately atmospheric humidity), a feedback that is affected by stomatal shape, density, and size. Stomatal shape varies throughout the plant kingdom, whilst monocots typically possess dumbbell-shaped stomata aligned in rows, dicots' kidney-shaped stomata are scattered across the leaf surface. When soil water is limiting dumbbell-shaped stomata close more rapidly compared to kidney-shaped (Hetherington and Woodward, 2003). This dynamic control in dumbbell stomata is influenced by the alignment of subsidiary cells parallel to guard cells, allowing extensive lateral movement during stomatal opening (Franks and Farquhar, 2006), the shape of dumbbell stomata also requires fewer solutes and less water to open the same aperture size as a kidney-shaped alternative,

making them more efficient (Atwell, Kreidermann and Turnbull, 1999). As such, this dynamic dumbbell stomatal control could have aided the diversification and spread of grasses during a time of global aridification 35 to 40 Ma ago (Hetherington and Woodward, 2003). At present day, and looking ahead to the future, these water-conserving traits, and dynamic responsiveness are valuable to plants experiencing low or fluctuating humidity conditions, and very relevant considering our reliance on monocot cereal crops (Hetherington and Woodward, 2003*;* Chen *et al*, 2017; Shtein *et al*, 2017).

As well as shape, the density and size of stomata also influence stomatal conductance, affecting overall plant productivity. Higher stomatal densities increase the total pore area and subsequent stomatal conductance. Since most monocots have dumbbell-shaped stomata, aligned in rows, higher stomatal densities are possible, resulting in a greater pore area per leaf area (Franks and Farquhar, 2006). Under fluctuating humidity conditions, these higher stomatal densities can be beneficial due to greater VPD sensitivity (El-Sharkawy, Cock and Del Pilar Hernandez, 1985), meaning rapid closure during low humidity, while allowing higher stomatal conductance in high humidity (Franks and Farquhar, 2006). In addition, increased stomatal density has resulted from high humidity conditions in rose plants (Torre and Fjeld, 2001; Arve *et al.*, 2013) as well as in tomato (*Lycopersicon esculentum* Mill.), eggplant (*Solanum melongena* L.) and sweet pepper (*Capsicum annuum* L.) when grown at high humidity (80% RH) compared to lower humidity (45%) (Bakker, 1991). In addition, some observations show, high humidity conditions increase stomatal aperture, resulting in higher stomatal conductance (Arve *et al.*, 2013) and plant growth (Jeon *et al.*,

2006) in Rose and *Doritaenopsis* respectively. Consequently, high stomatal density often goes hand in hand with smaller stomata, which are widely considered to respond more rapidly than larger stomata (Hetherington and Woodward, 2003). As such, smaller, denser, dumbbell-shaped stomata could be selected for breeding practices to be able to respond to changing humidity and soil moisture contents with greater efficiency. Whilst stomatal response to changes in humidity ultimately dictates the passage of carbon dioxide into and water out of the leaf, other morphological adaptations on the leaf surface can significantly affect this process, influences leaf wettability and leaf cuticle permeability.

1.3.2 Cuticle resistance

On the frontline alongside stomata, at the plant-atmosphere interface, is the leaf cuticle. This protective lipophilic membrane facilitated plants invasion of the land in the early Palaeozoic, over 400Ma (Dominguez, Heredia-Guerrero and Heredia, 2011; Renault *et al.*, 2017; Salminen *et al.*, 2018). The main function of the cuticle is to prevent against desiccation (Sánchez *et al.*, 2001), together with the governance of gas exchange (Littlejohn *et al.*, 2015) through cuticular transpiration (Kerstiens, 1996) alongside stomata, the cuticle also fulfils several other important roles including light reflection, heat tolerance, protection against mechanical injury from the environment, and some pathogens and pests (Dominguez, Heredia-Guerrero and Heredia, 2011). For a comprehensive review on the biophysical design of plant, cuticles see Dominguez, Heredia-Guerrero and Heredia (2011).

When humidity levels are high, water pooling on the leaf can create a water film across the leaf surface, blocking stomata (Vesala *et al.*, 2017), reducing stomatal

conductance (Hanba, Moriya and Kimura, 2004) and subsequently lowering net photosynthesis (Ishibashi and Terashima, 1995), these water films can, however, provide a valuable water subsidy in low soil moisture environments through the encouragement of foliar water uptake. Epicuticular wax affects the wettability of leaves and can, therefore, help to either pool water on the leaf surface leading to water film formation or encourage runoff via hydrophobic properties.

Epicuticular wax is comprised of a complex mix of organic compounds including long-chain alkenes, acetals, esters and acids (Eglinton and Hamilton, 1967) resulting in strong hydrophobic properties (Schuster *et al.*, 2016; Konnerup *et al.*, 2017) as such, it is good at preventing cuticular water loss (Jordan *et al.*, 1984; Ristic and Jenks, 2002; Schuster *et al.*, 2016) during low humidity conditions and is considered a xeromorphic property (belonging to plants growing in dry environments requiring little water for survival) (Oliveira, Meirelles and Salatino, 2003). Another adaptation to prevent excessive water loss is the acquisition of stomatal chimneys. These wax chimneys can rise above the stomatal pore, funnelling the air between the external atmosphere and stomatal pore interface, increasing the boundary layer resistance for stomatal conductance (Müller *et al.*, 2017). They are found in species from dry environments, such as *Phoenix dactylifera* (Müller *et al.*, 2017) and semi-arid *Euphorbia tirucalli* L. (Barthlott *et al.*, 1998).

When humidity is high enough for water to condense on the leaves, epicuticular wax can also be beneficial as the dense arrangements of wax crystals can allow air spaces to be maintained beneath water droplets (Barthlott and Neinhuis, 1997),

enabling stomatal conductance to continue. These gas films are also retained on the leaf surface for longer during complete submergence of superhydrophobic leaf surfaces compared to wax-less leaves (gas film minimum retention of four days and two days respectively) (Konnerup *et al*, 2017). Even though the presence of epicuticular wax is typically associated with plants adapted to arid environments, this morphological adaptation could also benefit crops experiencing both high humidity and flooding events. Epicuticular wax is also important for reducing conductance via wax plugs. Common in conifers (Brodribb and Hill, 1997), wax plugs prevent water film formation which can significantly hinder gas exchange over the pore (Feild *et al.*, 1998; Roth-Nebelsick, 2007) and potentially increase fungal pathogen load. However, under low humidity, wax plugs could be detrimental. When exposed to conditions with higher evaporative demand (~40%), *Drimys winteri*, a tree species common in wet forests, exhibit accelerated water loss in leaves with wax plugs than leaves where the plugs were experimentally removed (Feild *et al.*, 1998).

It is important to know the epicuticular wax properties of a leaf and how it could influence leaf wettability and plant responses to different levels of humidity. During a leaf wetting study (Hanba, Moriya and Kimura, 2004) on wettable bean plants (*Phaseolus vulgaris*), after a 72 h misting, plants experienced a 16% reduction in stomatal conductance and 55% reduction in the amount of Rubisco present. However, if a plant contains morphological adaptations that reduce the wettability of the leaves, the misting can have a positive impact on productivity. In the same study, a non-wettable pea (*Pisum sativum*) experienced a 12.5% increase in stomatal conductance after the misting event. Thus, suggesting the

effects of high humidity and potential water film formation differ between species, depending on their morphological adaptations. In this experiment, the pea plant (*Pisum sativum*) smooth waxy surface, proved difficult to wet, but benefits from the reduction in VPD and subsequently reduced transpiration. Whereas the bean plant (*Phaseolus vulgaris*), has a broad flat surface which was easily wetted (Hanba, Moriya and Kimura, 2004). Therefore, if plants possess specific morphological adaptations and have evolved to harness this resource, water films can be a valuable water subsidy and a physical barrier to significant water loss.

To uncover the chemical cuticular properties that will affect the wettability of a leaf and other physical properties, we can investigate the spectral chemical 'fingerprint' using attenuated total reflectance Fourier Transform infrared (ATR-FTIR) (Ribeiro da Luz, 2006). ATR-FTIR is an effective, non-invasive technique that involves the application of infrared energy to a sample from a global light source, molecules in the sample absorb the energy, exciting them from ground state to vibrational state, which results in a characteristic spectrum (Baker *et al.*, 2014; Liu and Yu, 2016; Liu *et al.*, 2019). Table 1 highlights the wavebands discussed in the literature alongside their associations with cuticular properties and compounds.

Table 1 Table highlighting wavebands of interest and their associations with cuticular chemistry and cuticular properties, as discussed in the literature.

	Waveband (cm^{-1})	Associated With	Reference
<1500 cm^{-1} Fingerprint region	725	Fatty compounds of the cuticle, mainly present in cutin and waxes	(Heredia-Guerrero *et al.*, 2016)
	783	N-H bending (amide V band of proteins)	(Ribeiro da Luz, 2006)
	989	C-O bending (cellulose)	(Ribeiro da Luz, 2006)
	1013	Pectin	(Ribeiro da Luz, 2006)
	1030 (broad peak)	Cuticular polysaccharides	(Johnson *et al.*, 2007) (Heredia-Guerrero *et al.*, 2016)
	1126	C-O stretching (polysaccharides)	(Ribeiro da Luz, 2006)
	1315	Fatty compounds of the cuticle, mainly present in cutin and waxes	(Heredia-Guerrero *et al.*, 2016)
	1458	O-H bending, assigned to cellulose functional groups	(Ribeiro da Luz, 2006)
	1465	Fatty compounds of the cuticle, mainly present in cutin and waxes	(Heredia-Guerrero *et al.*, 2016)
	1635	Ionised carboxyl groups	(Johnson *et al.*, 2007)
	1650	Water	(Liu *et al.*, 2019)
	1705, 1715, 1730	Cutin matrix waxes	(Heredia-Guerrero *et al.*, 2016)
	1736	C=O	(Liu *et al.*, 2019)
	1718-1750	Carbonyl ester region	(Liu *et al.*, 2019)
	2800-2950	Waxes have significant absorbance here due to symmetrical and asymmetrical stretching	(Liu *et al.*, 2019)
	2820-2866 (peak 2848)	CH_2 symmetric	(Heredia-Guerrero *et al.*, 2016)
	2855-2945 (peak 2918)	CH_2 asymmetric	(Heredia-Guerrero *et al.*, 2016)
	2954	CH_3	(Liu *et al.*, 2019)
	3400 region	Stretching mode of hydroxyl bonds. Main contributors are polysaccharides and non-esterified hydroxyl groups of cutin.	Heredia-Guerrero *et al.*, 2016)

Alongside the mechanical barriers of stomata and leaf cuticles, lies another significant layer -the boundary layer -which is affected by both environmental conditions such as humidity, but also morphological characteristics on the leaf surface.

1.3.3 Boundary Layer Resistance

The boundary layer is a blanket of 'calm' air surrounding the leaf and due to molecular binding of water to the leaf surface, is difficult to break. For transpiration to occur water must diffuse through this layer, which can be influenced by the external environment and cuticular properties. One way to reduce the VPD between the leaf environment and the external atmosphere is to create a thick boundary layer which is more humid than the atmosphere, therefore reducing stomatal conductance. Plants can influence the boundary layer in numerous ways including creating pits or crypts in which stomata reside, increasing trichome abundance on leaf surfaces, and building wax structures to trap air close to the leaf surface. Whilst a thick boundary layer is beneficial to plant in low humidity (high VPD) environments, during high humidity conditions, a thicker layer could present an even greater hindrance to stomatal conductance, leading to potential overheating and possible carbon starvation. Therefore, water repellent properties and morphological adaptations to reduce boundary layer resistance will be beneficial to plants growing in high humidity conditions, whereby leaves are vulnerable to overheating due to lack of transpirational cooling. However, plants that are capable of harnessing foliar water uptake for a valuable water subsidy, may promote adaptations that increase the boundary layer thickness, encouraging the diffusion of water vapour directly into the sub-stomatal

cavity or directly water pooling on leaf and subsequent cuticle diffusion (Schreel and Steppe, 2020).

1.3.3.1 Sunken stomata

In low humidity environments, increasing the boundary layer through sunken stomata is an advantageous morphological adaptation (Roychoudhury and Tripathi, 2019). Sunken stomata are set back from adjacent epidermal cells (Reef and Lovelock, 2015), or in crypts containing multiple stomata (Roth-Nebelsick, 2007), and are common in plants growing in dry environments (Mott and Peak, 2013). In a computer model, sunken stomata raised the local humidity around the pore space (Roth-Nebelsick, 2007) -in a similar fashion to stomatal chimneys- decreasing stomatal conductance and water loss (Poorter, 2004), a potentially beneficial adaptation during low humidity, high VPD conditions. However, this decrease in stomatal conductance could also be influenced by stomatal aperture as the maximum stomatal aperture decreases with increased stomatal depression depth (Lloyd and Woolhouse, 1978).

Though a trait generally associated with dry environments, sunken stomata could be beneficial to plants growing in high humidity conditions. A study on an endemic tree species (*Chamaecyparis obtusa* var. *formosana*) of the cloud forests of north-eastern Taiwan, found efficient photosynthetic processes were maintained despite the foggy conditions in which leaf surface moisture would be expected to hinder gas exchange, sunken stomata alongside stomatal clustering are thought to protect the stomatal pore from surface pooling water (Pariyar *et al.*, 2017), and subsequent blockage of stomatal pore. Therefore, sunken stomata

could be a beneficial trait for plants growing in both low and high humidity environments.

1.3.3.2 Trichomes

Trichomes are microscopic hairs protruding from leaves and stems, varying in size, structure and density, they are present in virtually all plant species (Glas *et al*, 2012). High trichome densities can enhance boundary layer thickness by trapping humid air around stomatal pores and reducing airflow across the surface of the leaf. As such, trichomes can reduce VPD across the epidermis and improve water conservation practices (Grammatikopoulos and Manetas, 1994; Nemeskéri *et al.*, 2009). Trichomes acting to increase the boundary layer resistance are therefore valuable properties of plants growing in low humidity environments, where water conservation is a priority (Lyshede, 1979; van der Merwe, van der Walt and Marais, 1994; Rotondi *et al.*, 2003), and could prove to be a valuable crop breeding trait, for unfavourably dry environments with low relative humidity. In addition to increasing the boundary layer resistance, dense trichomes can also reflect light (and heat) (Nemeskéri *et al.*, 2009) further supporting their potential value in hot, low humidity conditions. Examples of reflective trichomes can be found in soybean (*Glycine max* (L.) Merr,) (Du, Yu and Fu, 2009) and olive (*Olea europaea*) (Liakoura *et al*, 1997). In the air-plant species *Tillandsia,* trichomes increased the reflectivity of the leaf blade by 18-40% (Pierce, 2007). Whilst reflectance of up to 56% was observed from *Encelia farinosa* in the Sonoran desert of California (Ehleringer, Bjorkman and Mooney, 1976) Not only can trichomes be useful adaptations for low humidity environments, but they can also be valuable adaptations to plants growing in high humidity conditions. If trichome densities are high enough, the repellent properties of certain trichomes can prevent

water films from blocking stomata (Brewer *et al.*, 1997), thereby increasing transpiration rates (Palliotti, Bongi and Rocchi, 1994; Perez-Estrada *et al*., 2000). A study on 38 plant species across 21 families found trichome densities of more than 25mm^2 is needed to increase water repellency around stomata (Brewer, Smith and Vogelmann, 1991). As such, repellent trichomes are common features of cloud forest species such as the Bromeliaceae family (Pierce *et al*, 2001) and aquatic species including *Salvinia auriculata* and *Pistia stratiotes* (Neinhuis and Barthlott, 1997). However, not all trichomes in high humidity environments repel water, some can assist in direct foliar uptake such as the bromeliad shield structures in environments where humidity is high but soil moisture is low, water-absorbing adaptations such as the bromeliad shield structures can help provide a valuable water subsidy (Pierce, 2007).

Trichomes are a valuable morphological adaptation to plants in both low and high humidity environments. The degree to which the different structures can influence water repulsion or retention should be understood to aid our understanding of whole-plant responses to changes in humidity and soil moisture conditions. Boundary layer resistance, along with stomatal morphology and cuticle resistance are all key facilitators of gas exchange, between the plant and the atmosphere. Understanding how all three could change, and the knock-on effects it could have on plant productivity, is therefore critical to accurately predict the effects of humidity and soil moisture on whole plant physiology.

1.4 LEAF ARCHITECTURE

There are myriad morphological adaptations on the leaf surface, affecting the boundary layer resistance, leaf temperature, subsequent gas exchange and

potential foliar water uptake. Ultimately, it is whole leaf architecture, that heavily influences the previously discussed processes, so it is important to acknowledge how leaf architecture affects plant properties such as leaf temperature and water film formation, which would have knock-on effects to processes such as photosynthesis and potential foliar water uptake.

1.4.1.1 Convective Cooling

Although plants can cool themselves through transpiration and subsequent evaporative cooling (Ball, Cowan and Farquhar, 1988), with a number possessing effective cooling morphological adaptations such as reflective trichomes (Liakoura *et al.*, 1997; Du, Yu and Fu, 2009), one of the most effecting cooling strategies a plant employs comes from leaf physiognomy. Leaf shape and size influence air movement across the surface, and subsequently the efficiency of convective cooling (Givnish and Vermeij, 1976). This process is driven by convection whereby warm air rises, creating air currents that draw in comparatively cooler air (Gates and Benedict, 1963) thus reducing leaf temperature. Small leaves are more efficient, due to the shorter distance between the warm leaf and the cooler edge, resulting in higher rates of convective cooling (Vogel, 1968; Yates *et al*, 2010). In addition, serrated leaf margins also aid cooling as they cause turbulent airflow this increasing convective cooling (Wolfe, 1993) (Figure 3).

In dry environments, small, serrated leaves are preferential, with enhanced convective cooling and lower leaf area reducing the total stomatal pore area and subsequent transpirational water loss (Spicer, 2000). This leaf type could fare better in changing environmental conditions whereby humidity declines (higher

VPD), and/or soil moisture becomes limiting. Moreover, warmer leaves with higher rates of transpiration can benefit from evaporative cooling which can help to minimise further increases in leaf temperature, offsetting the evaporative demand to some extent (Ball, Cowan and Farquhar, 1988). Though during conditions with reduced transpirational demand e.g. high humidity (low VPD), transpirational cooling may not be adequate, which could cause a subsequent rise in leaf temperature which could have knock-on effects to photosynthetic processes.

A significant increase in leaf temperature causes a rise in transpiration (Nelson and Bugbee, 2015), triggering changes to the water potential gradient between the internal leaf environment and the external atmosphere. If a plant is unable to cool effectively, increased temperature could lead to a reduction in productivity as high leaf temperatures can lead to inactivation of Rubisco resulting in a decrease in net photosynthesis (Salvucci and Crafts-Brandner, 2004). Rubisco inactivation temperature varies between species e.g, Rubisco inhibition was observed at 35°C in Cotton (*Gossypium hirsutum* L.) and 30°C in wheat (*Triticum aestivum* L.) (Feller, Crafts-Brandner and Salvucci, 1998) and 32.5°C in maize (Zea mays) (Crafts-Brandner and Salvucci, 2002). Plants must therefore be able to cool themselves effectively so not to hinder photosynthesis and other biological processes.

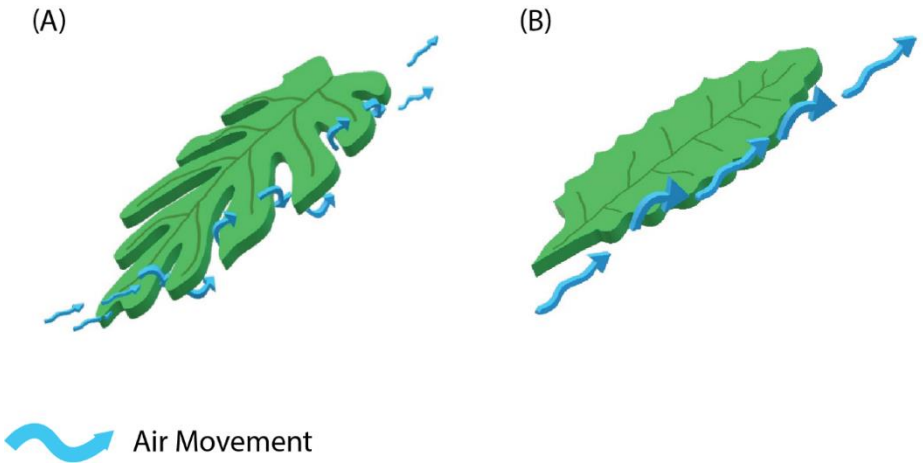


Figure 3. Schematic representing the effects of leaf shape on air movement and subsequent convective cooling. When leaves are large, increased dissection (A) encourages turbulent airflow and increased convective cooling surrounding the leaf. Serrated leaves (B) can encourage some turbulent airflow across the leaf margin, aiding convective cooling.

During high humidity conditions, plants have been observed to maximise their photosynthetic capacity by increasing leaf surface area (Spicer, 2000; Hovenden, Vander Schoor and Osanai, 2012), with large leaves a common occurrence in humid ecosystems. One drawback of large leaves is the distance between the warm leaf and cooler edge, which is why many become highly dissected to aid cooling via convection (Wolfe, 1993) (Figure 3). If leaves are unable to respond to high humidity conditions by altering their architecture, they are at a greater risk of overheating, a problem that could face broadleaved species if their environments become more humid, as predicted with global climate change in areas such as central and eastern United States, western China (Dai, 2006) and central Europe (Jones and Moberg, 2003).

1.4.1.2 Water Drips, Pooling, and Uptake

Leaf shape not only affects internal plant temperature, but can also influence water movement, pooling, and the capacity for foliar water uptake. During a precipitation event or when condensation exceeds the leaf storage capacity, the water can drip from the leaves, or run down the stem to the soil, both of which lead to increased soil moisture content.

Dawson (1998) estimated that 34% of the annual hydrology of the northern Californian region studied is attributed to fog drip. Whilst Stemflow provides water straight to the base of the plant where soil infiltration and interception by roots will be most effective (Carlisle, Brown and White, 1967). Furthermore, stemflow contains not only nutrients from precipitation but also nutrients leached from the vegetation (Eaton, Likens and Bormann, 1973) as such, stemflow return large quantities of nutrients (e.g. K and S) to the forest floor (Carlisle, Brown and White, 1967; Parker, 1983). Therefore, any modifications to leaf architecture will inevitably lead to changes in water and nutrient movement from the canopy to the root.

Drip tips are a common feature of high humidity, rainforest species, whereby the leaf bends downwards to a point thus promoting rapid water runoff (Lightbody, 1985) to the soil and roots below. This adaptation can also discourage water film formation and pooling on the leaves during precipitation events, which could hinder gas exchange by blocking stomatal pores. Though water retention on the leaf surface during high humidity environments could be detrimental to gas exchange, it may also provide a valuable water subsidy if the plant is capable of partaking in foliar water uptake (Limm *et al.*, 2009). In contrast, plants growing in

low humidity (high VPD) environments could benefit from rounded leaf tips and serrated margins, that help retard water dissipation, encouraging water pooling and thus increasing the boundary layer resistance and lowing VPD across the leaf surface.

1.5 PLANT-SOIL INTERFACE

In the traditional SPAC, transpirational demand places a large pressure on soil derived water supply, as such, normal growth and development is dependent on the plant's ability to explore soil resources. The metabolic costs of soil exploration can exceed half of daily photosynthesis (Lambers, Atkin and Millenaar, 2002b), therefore efficient root systems are key for maintaining productivity in sub-optimal (e.g. dry) soil conditions. Both root anatomy and root system architecture are key determinants of root water uptake and can display a great deal of phenotypic plasticity towards changing environmental conditions (Zhu *et al.*, 2011; Lynch, 2015).

1.5.1 Root Anatomy

The main pathway for extensive water flow from the roots to aerial organs is the xylem. The radial movement of water (Figure 4) across the root takes place through cortical tissue, taking a partly apoplastic (movement through the water-filled free space of cell walls) and partly symplastic (in the protoplasm of the cell membrane) approach (Jones, 2013). The exact proportion of apoplastic to symplastic contributions is difficult to calculate due to anatomical complexity and

varying permeability of cell membranes (Jones, 2013).

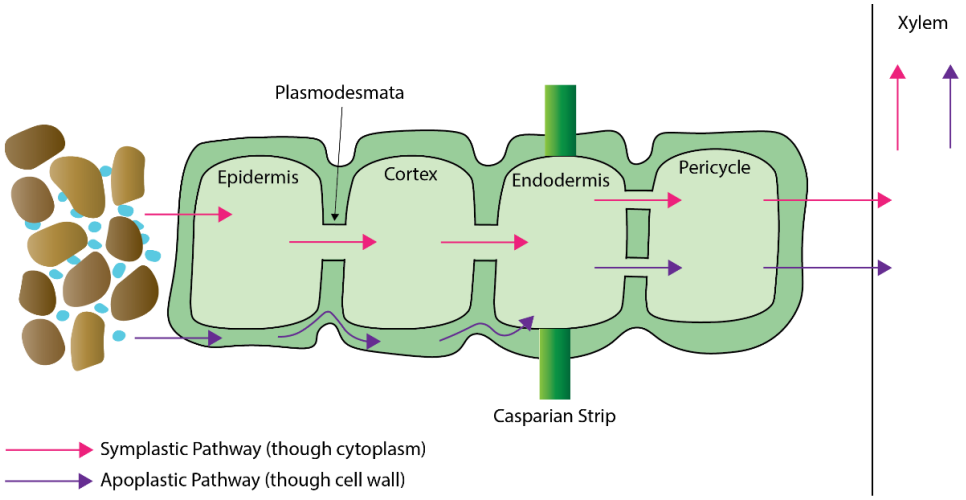


Figure 4. A diagram displaying the difference between symplastic and apoplastic pathways of water uptake from the soil through the root into the xylem.

Root anatomy influences the flow from the rhizosphere, across the root and into the xylem, as well as affecting the overall metabolic costs of root growth and exploration. The number of cortical cell files directly influences the distance water and other soluble nutrients must travel from the soil to the xylem. During drought conditions, plants can reduce the cortical cell file number (CCFN) to reduce the distance travelled to the xylem and to also reduce the metabolic costs of soil exploration, thus improving drought tolerance (Chimungu, Brown and Lynch, 2014; Vadez, 2014). Moreover, fewer cortical cells that are larger in diameter are also favoured for their lower metabolic demands (Colombi *et al.*, 2019). Such changes to root anatomy not only affect the plant at the root level but can also have significant impacts on above-ground organs and processes. A study on maize also found that genotypes with lower CCFN, exhibited significantly higher rates of stomatal conductance, greater leaf carbon assimilation and higher shoot biomass, compared to genotypes with many cortical cell files (Chimungu, Brown

and Lynch, 2014). In extreme drought conditions, plants can function without cortical cells through the process of cortical senescence (also known as root cortical death) as is a common phenomenon in cereals, particularly wheat and barley. Cortical senescence results in the outer layers of the root to be completely lost, leaving only the endodermis and the stele (Lynch, Chimungu and Brown, 2014). This rather extreme response reduces both the diffusional distances that water and nutrients need to travel and also the metabolic costs of root growth due to the loss of living tissue (Lynch, Chimungu and Brown, 2014).

Another key anatomical trait that affects resistance and water movement through roots is aerenchyma, and both low and high soil moisture conditions can induce root aerenchyma formation, to help overcome two very different environmental stresses. Aerenchyma are tissues containing air spaces that can affect both oxygen and water transport. During waterlogged conditions, aerenchyma act as conduits transporting oxygen from the aerial organs to the hypoxic roots (Mohammed *et al.*, 2019). Conversely, amidst drought conditions, root cortical aerenchyma permits greater drought tolerance in maize through the reduced metabolic cost of soil exploration (Chimungu *et al.*, 2015), allowing greater root growth and water uptake (Zhu, Brown and Lynch, 2010).

Comparable to changes in cortical characteristics, aerenchyma formation can have significant knock-on effects on aerial organs and processes. For example, maize genotypes with high levels of root cortical aerenchyma formation, have been found to exhibit greater leaf water content, shoot biomass and grain yield, compared to low levels of aerenchyma forming genotypes under drought

conditions (Chimungu *et al.*, 2015). Furthermore, changes to stele root anatomy can also influence water movement and metabolic costs and soil exploration. An increase in stele diameter is considered advantageous in hard soils, increasing root penetrability (Chimungu, Loades and Lynch, 2015; Klein *et al.*, 2020). This could be beneficial for plants growing in low soil moisture conditions as soil strength (regarded here as the mechanical resistance to root penetration through the soil) increases non-linearly with decreasing soil moisture. Therefore, increased stele diameters could be a beneficial trait for plants growing in low soil moisture conditions, in terms of overcoming the mechanical impedance and furthering soil exploration efforts for valuable water resources. During low soil moisture and high transpirational demand (caused by high VPD) conditions, plants are at risk of xylem cavitation and embolism (Sperry and Sullivan, 1992). This danger occurs when xylem water potentials drop below the xylem-specific threshold, enabling air to enter the conduit from adjacent air-filled cells, thus interrupting capillary action (Brodribb, McAdam and Carins Murphy, 2017) and leading to cavitation (Zimmermann, 2013). By reducing the number of xylem vessels (Henry *et al.*, 2012) and/or size (Fichot *et al.*, 2009), reduces the area available for cavitation to occur and concentrates transpiration streams. Plants that can lower their hydraulic requirements are considered to be more water-use efficient and better equipped to cope with drought stress conditions (Fichot *et al.*, 2009) and reduced metaxylem size in wheat has been linked to increased yields during low soil moisture conditions (Richards and Passioura, 1989).

Changes to root anatomy therefore significantly influence water uptake, and transport around the plant, as well as affecting the metabolic costs of root growth

and development. These changes will inevitably affect the plant's ability to explore the soil environment, thus affecting the whole root system architecture.

1.5.2 Root System Architecture

Root system architecture (RSA) refers to the spatial arrangement of roots from a single plant (Zhu *et al*., 2011; Lynch, 2013). Only a small number of roots are produced during embryogenesis, with most of the root system establishing itself as the plant grows. The RSA, therefore, exhibits plasticity and dynamic response to environmental stresses (Hepworth, *et al.*, 2016) e.g. soil moisture availability and is, therefore, a good indicator of prevailing environmental conditions (Zhu *et al*., 2011) both below and above the ground due to extensive communication between the subterranean and aerial organs (Nibau, Gibbs and Coates, 2008). As such, there is a well-established relationship between root system architecture and crop performance (Zhu *et al*., 2011).

The procurement of soil resources is a fundamental limitation to crop production (Lynch, 2013) and a modelling study on the US Corn Belt (Hammer *et al.*, 2009) indicated that the RSA and subsequent acquisition of resources were more important than canopy architecture and subsequent capture of light, with regards to biomass production and high yields when grown at high densities. Thus highlighting the importance of studying RSA and understanding its function during resource limiting conditions (Manschadi *et al.*, 2006). Such knowledge could aid the development of new crop cultivars with enhanced root foraging capacity and resource acquisition in sub-optimal conditions. A strategic goal, as we face resource depletion, climate change, and a surging global population.

An efficient soil resource acquisition strategy is one that maximises resources uptake whilst reducing carbon costs (Nibau, Gibbs and Coates, 2008; Alvarez-Flores *et al.*, 2014), as such, plants have adopted myriad morphological responses. Whilst steeper growth angles and deeper root penetration can help unlock previously inaccessible soil water reserves (Bauerle *et al.*, 2008; Nibau, Gibbs and Coates, 2008; Henry *et al.*, 2012), shallower rooting strategies are beneficial to plants growing in phosphorous limiting environments due to the immobility of the nutrient, resulting in confinement to the upper layers. The surface area of RSA can be increased through accelerated lateral root formation and higher root hair densities (Nibau, Gibbs and Coates, 2008; Hepworth *et al*., 2016), increasing the extent of the soil-root interface (rhizosphere) and enhancing resource uptake (Lambers, Atkin and Millenaar, 2002). For a comprehensive review on root system traits and their subsequent effects on water and nutrient acquisition see Lynch (2013).

Though root responses are not solely confined to belowground conditions. The effects of atmospheric CO_2 concentrations on root growth and development have been observed in a range of species. A study on *Senecio vulgaris* found elevated CO_2 concentrations (700 μmol mol^{-1}) resulted in longer, more heavily branched roots with a greater foraging capacity (Henry *et al.*, 2011). Whilst investigations on Soybean (*Glycine max* L. Merr 'Lee' nonnodulating) (Rogers *et al.*, 1992), grown at CO_2 concentrations of 350 and 700 μmol mol^{-1} found not only increased root length and root dry weight at high CO_2 concentrations but also changes to root anatomy with increased stele diameter and cortex width resulting in increased root diameter (Rogers *et al.*, 1992). Such effects are somewhat expected, as during

elevated atmospheric CO_2 there is more carbon available for plant growth and development. However, the influence of atmospheric CO_2 on root responses may also depend on other external factors such as nitrogen availability in the soil. A study on longleaf pine (*Pinus palustris* Mill.) at 365 and 720 µmol mol^{-1} (Prior *et al.*, 1997), found increased biomass production, but only in the high nitrogen treatment. Furthermore, belowground responses are also intrinsically linked to aboveground processes, which can also be influenced by a range of environmental conditions. More recently, Hepworth *et al.*, (2016) found that *Arabidopsis thaliana* with higher stomatal densities and stomatal conductance exhibited a larger rooting area, resulting in greater phosphate uptake capacity, whereas low stomatal conductance and lower stomatal densities resulted in smaller root systems.

Morphological changes to root anatomy and architecture not only reflect the belowground environment, but also aboveground conditions. Aerial and subterranean organs are intrinsically linked and so too are their responses to perturbations in environmental conditions. Through studying such responses, we can begin to understand the implications and attempt to decouple the impacts of humidity and soil moisture on whole plant physiology. However, whole-plant physiological responses are not only affected by differing physiological adaptations and morphologies at the plant-atmosphere and plant-soil interface but also on inherent plant behaviours. These deep-rooted behaviours e.g. different photosynthetic strategies (C3, C4 and CAM) or levels of isohydricity will inevitably alter a plant's sensitivity to changing conditions, including humidity and soil moisture.

1.6 WHOLE PLANT SENSITIVITY

1.6.1 Photosynthetic Strategy

Plants exhibit one of three photosynthetic strategies: C3, C4, and CAM (with a few intermediates noted). The three processes differ in their carbon fixation chemistry and anatomy with photosynthetic type depending largely on adaptation and habitat (Cousins, Mullendore and Sonawane, 2020). Briefly, C3 photosynthesis, the prevailing mechanism found in cereals in temperate and Mediterranean regions (in plants such as wheat and rice), produces a three carbon-compound (3-phosphoglyceric acid) via the Calvin-Benson Cycle as the first step in C fixation (Cousins, Mullendore and Sonawane, 2020). The more evolutionary recent C4 photosynthesis, found in warmer, tropical and arid regions (in plants such as maize), initially produces a four-carbon intermediate (Malate) which then splits to produce three-carbon compound for the Calvin cycle, the latter normally occurring in a second specialised cell to enable CO_2 concentration and reduce or eliminate photorespiration (Cousins, Mullendore and Sonawane, 2020). CAM (crassulacean acid metabolism) are succulent plants (for example, pineapple), common in arid and desert regions, fix carbon at night and close stomata during the day (Cousins, Mullendore and Sonawane, 2020). The key points to remember are that C3 plants will operate photorespiration at high temperatures because they are unable to prevent oxygenation of the initial substrate RuBP but this is less limiting at low temperatures. In warm regions where photorespiration can seriously limit growth rates, C4 plants can reduce or eliminate it by concentrating CO_2 around Rubisco, suppressing the oxygenation reaction. CAM plants, such as cacti, are so severely water-limited that they have evolved a metabolically costly but water-conserving mechanism whereby they open stomata in the cool humid

night and use starch reserves to power CO_2 fixation by Phosphoenolpyruvate carboxylase into malic acid which is then decarboxylated and enters the Calvin cycle during the day, using solar energy when stomata are shut (Cousins, Mullendore and Sonawane, 2020).

Typical differences in characteristics of C3, C4, and CAM photosynthetic pathways are presented in Table 2. For a comprehensive explanation of the biochemical and physiological differences between C3, C4, and CAM photosynthetic pathways see Jones, (2013). The type of photosynthetic strategy that a plant adopts will significantly influence the plant water relations and the sensitivity of a plant to changing environmental conditions. For example, C4 plants have a higher water use efficiency (Way *et al.*, 2014), than C3 plants, and are therefore considered more drought-tolerant, so changes to soil water content may not affect a C4 plant as much as a C3. Also, C4 plants have more 'sensitive' stomata, so changes to VPD (through changing humidity levels), may cause a greater response to the more perceptive C4 plants, finely tuned to their aerial environments, compared to the more sluggish C3 stomata. A comparative study (Nayyar and Gupta, 2006) on the differential sensitivity of water-deficit stress in wheat (*Triticum aestivum*) and maize (*Zea mays*), which represented C3 and C4 photosynthetic strategies, respectively, showed that under moderate to high water stress, C3 plants showed considerably more water loss and oxidative stress compared to C4 plants.

Understanding how the photosynthetic strategy may alter a plants 'sensitivity' to above and belowground conditions, could help us predict which plants may be more affected by perturbations to environmental conditions e.g. soil moisture,

humidity (VPD), and identify key strategies (e.g. C3 vs C4) for increasing productivity during our unstable climate.

Table 2. Characteristics of C3 and C4 photosynthetic pathways. Table adapted from information in (Jones, 2013)

Characteristic	**C3**	**C4**	**CAM**	
			Day	**Night**
Relative stomatal sensitivity to the environment	Insensitive	Sensitive	Reversed (closed during the day, open at night)	
Optimum day temperature	15-30 (wide acclimation)	25-40	~35°C (requires low night temperature)	
Light response saturating well below full sunlight	Usually	Rarely	Usually	-
Most common growth region	Temperate	Tropical, Arid	Arid	
Transpiration ratio (g H_20 lost/g CO_2 fixed)	High 450-950	Low 250-350	Medium (50-600)	Very Low (<50)
Max. growth rate (g m^{-2} day^{-1})	34-39	51-54	7	
Average productivity (tonne ha^{-1} yr^{-1})	c.40	60-80	Low	

1.6.2 Isohydric and Anisohydric Behaviour

Alongside photosynthetic strategy, where a plant lies on the isohydric/anisohydric spectrum will significantly affect which environmental conditions cause more stress, the level of sensitivity to such stresses and the subsequent plant responses.

For a summary of the main differences between isohydric and anisohydric behaviour see Table 3. Briefly, isohydric plants maintain a constant midday leaf water potential through rapid stomatal control whereas anisohydric plants take a riskier strategy, allowing for decreasing midday leaf water potentials permitted by the slower stomatal response.

Table 3. Differences associated with true anisohydric and isohydric plant behaviour, when exposed to low soil water availability. Adapted from the information presented in Sade, Gebremedhin and Moshelion 2012 and Nolan *et al*. 2017. The term 'loose' stomatal control is referring to only closing stomata once the plant is under considerable water stress.

Factor (under low soil water availability condition)	**'True' Anisohydric**	**'True' Isohydric**
Midday leaf water potential	Decreasing	Constant
Stomatal conductance	Maintained	Reduced
CO_2 assimilation	Maintained	Reduced
Hydraulic vulnerability	Lower	Higher
Level of stomatal control	Loose	Tight
'Biggest' risk	Hydraulic failure	Carbon starvation

Some plants are extremely sensitive to changes in leaf water potential, with rapid stomatal closure to maintain constant water potentials within the leaf, these plants are known as isohydric (Klein, 2014). Examples of isohydric plants include maize

(Tardieu *et al*. 1993), pea (Bates and Hall, 1981) sugar maple (*Acer saccharum* Marsh.) and tulip poplar (*Liriodendron tulipifera* L.) (Yi *et al.*, 2017). At the other end of the spectrum are anisohydric plants, which are far less sensitive, allowing stomata to remain open for longer while the leaf water potential drops. Examples of anisohydric plants include wheat, soybean (Wilkinson, 2000) peach (Steinberg, Mcfarland and Miller, 1989) and oak species (*Quercus alba* L. and *Quercus rubra* L.) (Yi *et al.*, 2017).

Anisohydric behaviour is considered the riskier strategy, as the fluctuations in leaf water potential could result in irreversible hydraulic failure during conditions of rapid dehydration (Sade, Gebremedhin and Moshelion, 2012). In contrast, isohydric plants are more conservative and close stomata during drought conditions to maintain xylem pressures above -2.5 MPa (McDowell *et al*, 2008), making them less likely to experience cavitation and hydraulic failure (McDowell *et al*, 2008; Roman *et al*, 2015; Skelton, West and Dawson, 2015). Whilst isohydric behaviour can benefit plants growing in low humidity (high VPD) and/or low soil moisture conditions, reducing water loss through rapid stomatal closing, the prolonged closure of stomata can result in carbon starvation and a reduction in overall health and biomass production (McDowell *et al.*, 2008). As such, despite being the more risky strategy, anisohydric plants can maintain higher rates of photosynthesis under water stress (Attia *et al*, 2015), and have increased rates of night-time xylem refilling (Yi *et al.*, 2017) compared to isohydric plants and therefore respond more effectively to drought conditions (Linton, Sperry and Williams, 1998; Alsina *et al.*, 2002; Clearwater and Clark, 2003; Brodribb and Holbrook, 2004). In areas where humidity is predicted to

decrease, increasing the VPD and subsequent transpirational demand on the plant, anisohydric plants could fare better with regards to maintained productivity. However, there is currently little research directly looking at the effects of humidity on plant physiology whilst considering the effects of isohydric/anisohydric behaviour. Future humidity studies should acknowledge where on the isohydric spectrum their studied plants lie, as this will aid our understanding of behaviour strategies for different growth conditions, shedding light on beneficial traits to inform future breeding practices.

1.7 CONCLUSION

Plant water movement is complex and multidirectional and is affected by a plethora of morphological adaptations, which affect the resistance, transpirational demand and metabolic costs required of photosynthesis. The sensitivity of such adaptations to changing environmental condition is dictated by inherent photosynthetic and isohydric behaviours. As such changes to humidity and soil moisture will affect not only the supply and demand of water to the plant system, but also the morphological adaptations that facilitate or prevent water movement to maximise water use efficiency, photosynthesis, and ultimately, plant survival.

1.8 RESEARCH AIMS AND OBJECTIVES

The overarching aim of this research is to decouple the effects of humidity and soil moisture on whole plant physiology.

This project will specifically investigate shoot and root physiological responses to high and low humidity and soil moisture growth conditions in two key crop species maize (*Zea may*) and wheat (*Triticum aestivum* cv. Paragon). Through quantification of the effects on biomass production, stomatal morphology, gas exchange and chlorophyll fluorescence, ABA concentrations, root anatomy,

cuticular chemistry, and root system architecture we hope to achieve the following aims.

1. Identify whether high humidity can help alleviate low soil moisture stress (Chapters 2,3,4,5)
2. Investigate whether different species with different photosynthetic regimes (C3 and C4) have different levels of sensitivity to treatment conditions (Chapters 2,3,4,5).
3. Measure shoot and root responses to different humidity and soil moisture regimes (Chapters 2,3,4,5)
4. Utilise μCT technology for a detailed analysis of root system architecture responses to different humidity and soil moisture regimes (Chapter 5).
5. Investigate the effects of humidity and soil moisture on gas exchange and chlorophyll fluorescence parameters (Chapters 2 and 5).

1.9 CONTRIBUTION TO THE DISCIPLINE

Through the identification and quantification of maize and wheat physiological responses to humidity and soil moisture, we will gain a better insight into how crops and the environment interact. With an increasingly erratic climate, this insight will prove valuable to predict future responses to climate scenarios. This thesis will provide novel findings with regards to the decoupling of humidity and soil moisture responses, whilst highlighting the varying physiological responses between two key crops. As such, the research presented could help to improve crop management strategies, irrigation practices, and future breeding practices.

2 THE EFFECTS OF ATMOSPHERIC HUMIDITY AND SOIL MOISTURE ON STOMATAL PHYSIOLOGY, PHOTOSYNTHESIS AND BIOMASS PRODUCTION

2.1 INTRODUCTION

Changes in global humidity could significantly affect plant productivity, which would, in turn, affect crop yields and global food security. This is a significant issue as the global population is expected to reach 10 billion by 2050 (Truong, McCormick and Mullet, 2017), thus mounting pressure on food production systems. As it stands, global cereal crop productivity needs to increase by 39% to over 4 billion metric tons by 2050 (Shah and Wu, 2019). We not only need to understand how to improve crop productivity now but also predict and prepare for changes in productivity in response to perturbations in the climate. Understanding how plant functioning responds to environmental changes will aid the selection of advantageous traits that suit the prevailing environmental conditions. This has been the basis for the so called 'physiological breeding' programs operating in sites like CIMMYT, Mexico (Reynolds and Langridge, 2016).

Whilst the impacts of drought, temperature and light intensity on plant productivity has been extensively studied on crops such as maize and wheat (Tao *et al.*, 2016; Akter and Rafiqul Islam, 2017; Zhang *et al.*, 2018; Hussain *et al.*, 2019; Nisa *et al.*, 2019), one environmental factor that has been somewhat overlooked is that of humidity, which directly influences the vapour pressure deficit (VPD), the water potential gradient between the plant and the atmosphere, that drives processes such as transpiration. During commercial glasshouse growth environments, where the grower has some control of the environment, the optimal

VPD is generally considered to be below 1.5kPa (Shamshiri *et al.*, 2016), though it is not uncommon for VPD to be >2kPa, especially during the summer months (Lu *et al.*, 2015; D. Zhang *et al.*, 2018). An increase in VPD leads to an increase in transpirational demand. Shifts in the driving forces behind these processes could significantly affect not only global crop production in terms of transpirational water loss and abiotic stresses but also the hydrological cycle of affected ecosystems.

Humidity and subsequent VPD changes will influence water loss of major crop systems. A recent modelling effort of the US Corn Belt highlights the significance of VPD and soil moisture in regulating stomatal behaviour of maize and soybean (Kimm *et al.*, 2020). Through the analysis of eddy covariance data from seven sites across the US Corn Belt, the model attributes variability in canopy-level stomatal conductance and gross primary productivity to both VPD and soil water status (Kimm *et al.*, 2020). The significance of the effects of VPD on plant productivity is further endorsed by a study carried out by Novick *et al*. (2016). This modelling study concluded that low VPD is a greater stress to plants from a variety of biomes (evergreen forest, deciduous broadleaf forest, croplands, grasslands and savannahs and shrublands) than dry soil conditions. Such findings are further supported by Leuschner (2002) who concluded that VPD acts as a soil water independent growth controlling factor on temperate woodland herbs. Though these modelling and field studies emphasise the importance of VPD and attempt to decouple the effects of humidity and soil moisture on plant water relations, there is a lack of research at the individual plant level, investigating the effects of humidity and soil moisture on overall plant physiology, both above and

below the ground. We need to understand these processes and mechanisms if we are to identify relevant traits and generate more productive plants to help us achieve our targets for 2050 and beyond.

This chapter will investigate above and belowground physiological responses to humidity and soil moisture of two key crops, maize (*Zea mays*) and wheat (*Triticum aestivum* cv. Paragon), with the aim of decoupling these two factors. Maize and wheat were selected as appropriate for this study because they are major global crops, ranked 2nd and 4th (respectively) in terms of overall production (*FAOSTAT*, 2018), and important for global food security.

Maize (C4) and wheat (C3) represent different photosynthetic strategies which could influence their sensitivity to humidity and soil moisture treatment conditions. This is crucial as all crop-growing regions of both C3 and C4 plants will be affected by changes in humidity and soil moisture as a result of climate change. Maize (isohydric) and wheat (anisohydric) lie at opposite ends of the isohydricity spectrum, with differing levels of maintenance of midday leaf water potentials (McDowell *et al*, 2008) and stomatal control (Klein, 2014). Since humidity changes affect VPD which subsequently affects transpirational demand and leaf water potentials, a plant's position on the isohydricity spectrum could greatly affect its physiological response to the treatment conditions. Therefore, understanding how different species, photosynthetic strategies, and isohydric/anisohydric behaviour influence a plant's response to changing humidity and soil moisture conditions, could be beneficial to global crop productivity models.

Firstly, this chapter will investigate the treatment effects on stomata, the gatekeepers of the interface between the leaf and external environment. These adjustable pores dictate carbon gain and water loss (Jones, 1998; Brodribb and McAdam, 2011) thus influencing both plant productivity and plant water status. Stomata can respond to both soil water status via signalling from the roots (Dodd, 2005; Christmann *et al.*, 2007) and directly to changes in atmospheric humidity (Holbrook *et al*, 2002). Stomata could therefore provide an insight into plant perceptions of above (humidity) and belowground (soil moisture) treatment effects, shedding light on which is the dominant driver of changes to plant water status, and overall productivity. With regards to productivity, the chapter will then examine how a plant's photosynthetic ability is affected by treatment conditions, as reductions in photosynthetic capacity and efficiency have been linked to decreases in yield potentials (Murchie *et al*., 2009; Zheng *et al*., 2019; Zhu *et al*., 2010).

There are multiple options for assessing photosynthetic rate, stomatal conductance and transpiration in plants, the most obvious being infrared gas analysis (IRGA) which allows a direct measurement of both photosynthesis and water loss. Additionally, canopy temperature is important. Whilst open stomata and high transpiration rates are associated with high photosynthesis, transpirational cooling, stomatal can result in higher leaf temperatures with knock-on physiological effects (Janka *et al.*, 2013) such as the inactivation of critical photosynthetic enzymes such as Rubisco at higher leaf temperatures (Salvucci and Crafts-Brandner, 2004). Measuring leaf temperature, normally by IR sensors and

cameras can, therefore, aid our understanding of transpiration in the field and the potential for stress associated with stomatal closure, and how this could affect other processes such as photosynthesis. The use of IR thermography on crops as a method for rapid phenotyping for photosynthesis and biotic and abiotic stresses (Masuka *et al.*, 2012; Janka *et al.*, 2013; Prashar and Jones, 2014) has been gaining traction in recent years, aiding crop management systems and providing phenotypic information to aid crop breeding practices for particular environmental conditions.

The quantum yield of photosystem II (ΦPSII), is a common and easily made measurement from chlorophyll fluorescence techniques: this is an indication of the operational quantum yield of PSII in the light, the higher the ΦPSII the higher the efficiency, with potentially more energy being dissipated via photochemistry. A separate measurement gives the energy lost as heat (non-photochemical quenching, NPQ). When assessing the treatment effects on the photosynthetic capacity of a plant it is important to look at the ratio of light energy that is going towards non-photochemical quenching (NPQ). NPQ is a protective mechanism that regulates light use, prevents over – reduction of electron transport and limits the occurrence of damaging photoinhibitory mechanisms (Goss & Lepetit, 2015; Kromdijk *et al*., 2016; Murchie & Ruban, 2020).

Chlorophyll content was investigated as it is an important broad indicator of multiple physiological conditions, it can be affected by drought, humidity and leaf nitrogen status (Xiong *et al.*, 2015). Reductions in chlorophyll content are indicative of drought conditions (Li *et al.*, 2006; Mafakheri *et al.*, 2010; Arjenaki,

Jabbari and Morshedi, 2012), therefore, changes to relative chlorophyll content are considered a reliable screening indicator of drought tolerance (Li *et al.*, 2006; Mafakheri *et al.*, 2010; Arjenaki, Jabbari and Morshedi, 2012; Chen *et al.*, 2016). However, VPD can also affect chlorophyll content and therefore needs to be considered during such screenings. A study on barley (Sánchez-Díaz *et al.*, 2002) found that in plants growing under low soil moisture conditions, chlorophyll content remained higher in plants grown under high VPD compared to low VPD.

Lastly, this chapter will investigate the effects of these treatments on biomass, both above and below the ground to gain an understanding as to whether potential changes in stomatal morphology, photosynthesis and cell expansion in response to water deficit conditions (Gimenez, Gallardo and Thompson, 2004) are impacting plant growth and biomass production.

2.2 HYPOTHESES TO BE TESTED

This chapter will investigate whether humidity affects above and belowground physiology in species with different photosynthetic pathways and isohydric behaviours. It will investigate whether high humidity has the potential to mitigate the potential stress associated with low soil moisture conditions.

This chapter will test the following hypotheses:

1. Stomatal size will increase, and stomatal density will decrease in high humidity conditions

2. More stomata will be open in high humidity conditions compared to low humidity, regardless of soil moisture.

3. High humidity will increase ΦPSII values (higher photosynthetic capacity)

4. Shoot biomass (dry weight) will increase in high humidity

5. Root biomass (dry weight) will be unaffected by humidity.

2.3 MATERIALS AND METHODS

This experimental chapter was designed to test both experimental methods and sampling techniques. The chapter was therefore treated as an investigative pilot study whereby future experiments would be based around. Due to limited space and time available to carry out the following experiment, limited number of replicates were achieved, which may have influenced the statistical outcome. Findings from this chapter were therefore further investigated in Chapter 5, with more rigorous experimental design (larger number of replicates and more controlled growth conditions) as well as more in depth investigation of plant physiology (e.g. root architecture analysis from CT scans and gas exchange measurements with a Licor Infrared Gas Analyser). The results from this chapter helped to guide future experimental chapters as well as infer some possible humidity and soil moisture treatment effects on both maize and wheat.

2.3.1 Plant Material and Experimental Design

12 maize (*Zea mays*) and 12 wheat (*Triticum aestivum* cv. Paragon) seeds were germinated in a polypropylene column (height 25.5cm, radius 4cm), packed with a 50:50 mix of sandy loam and sand at a bulk density of 1.3 g/cm^3.

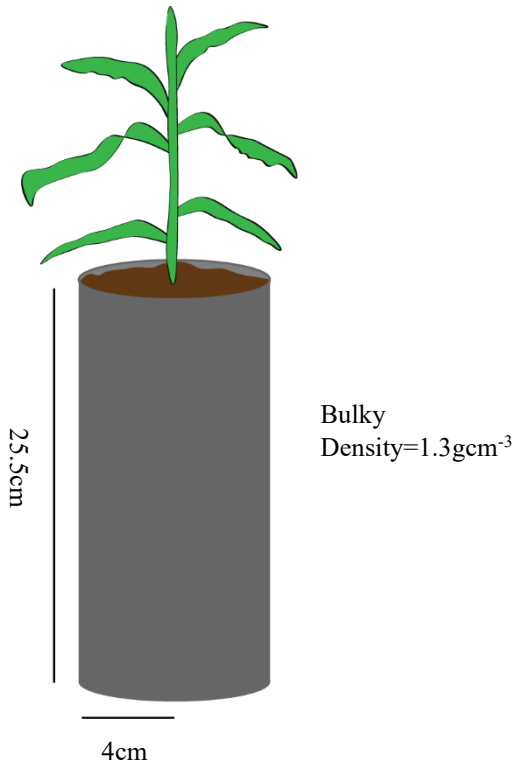


Figure 1. Maize and wheat plants were germinated and grown in polypropylene columns suitable for CT scanning.

The sandy loam soil was collected from a field site in Bunny, Leicestershire, UK (Longitude=-1.12608866, Latitude=52.86098725).

During germination soil moisture for both treatments was maintained at 70% field capacity. Once germinated, the columns were randomly arranged in a custom chamber that was situated in a temperature-controlled (heating and vents)

glasshouse. The chamber was constructed as a 4 × 4 m wooden frame, covered in opaque plastic sheeting to ensure equal light distribution throughout the chamber (though exact light levels were not measured), as well as containing the fog during high humidity conditions. The chamber was split into two sub-chambers divided by the same plastic sheeting where one side remained at low humidity whilst the other contained a humidifier (Nordcel ultrasonic 5L humidifier, CF-2756H) supplied with deionised water.

Figure 2. Custom growth chamber inside the glasshouse. Low humidity conditions maintained on the left-hand side, and high humidity on the right. Humidity was provided by a 5L ultrasonic humidifier (Nordcel, CF-2756H). Opaque plastic sheeting surrounded both sides of the chamber to ensure equal light distribution throughout the chamber.

Three reps of maize and wheat were randomly arranged in each sub-chamber and were subjected to the following treatment conditions (Table 1). Soil moisture was maintained with regular weighing and watering. Air temperature and humidity were recorded every hour in either side of the growth chamber (high and low humidity compartments), using a Fisher Scientific Traceable Humidity/Temperature. Dew-Point Meter (Fisher, UK), daily average recorded values are presented in Figure 3. Vapour pressure deficit (VPD) values were calculated using recorded air temperature and humidity data. Air vapour pressure deficit was calculated using the following two equations from (Jones, 1992)

$$Saturation\ Vapour\ Pressure\ (SVP)\ = 610.78 \times e^{\frac{T}{(T+238.3)\times 17.2694}} \qquad Equation\ 1$$

Where *T* is degrees in Celsius, *e* is the mathematical constant Euler's Number, approximately equal to 2.71828. The result *SVP* is in pascals and was dived by 1000 to get kPa

$$Vapour\ Pressure\ Deficit\ (kPa) = SVP \times \left(1 - \frac{RH}{100}\right) \qquad Equation\ 2$$

Where *SVP* is the calculate saturation vapour pressure from Equation 1 and *RH* is relative humidity (%).

Table 1. Treatment growth conditions. Relative humidity and temperature averages for the experimental period are shown. Measurements were made using a Fisher Scientific Traceable Humidity/Temperature. Dew-Point Meter (Fisher, UK). Day refers to 06:00 – 18:00 and night (18:01 – 05:59). Soil moisture treatment was maintained with regular watering to weight (gravimetric measurements).

Treatment	**Relative Humidity (%) (day/night)**	**Soil moisture (field capacity %)**	**Temperature °C (day/night)**	**Calculated Air Vapour Pressure Deficit (VPD_{air} kPa) (day/night)**
High Humidity High Soil Moisture **(HHHS)**	92.1/99.9	70	29.3/20.8	0.50/0.02
High Humidity Low Soil Moisture **(HHLS)**	92.1/99.9	30	29.3/20.8	0.50/0.02
Low Humidity High Soil Moisture **(LHHS)**	29.8/48.0	70	30.5/21.3	3.55/1.32
Low Humidity Low Soil Moisture **(LHLS)**	29.8/48.0	30	30.5/21.3	3.55/1.32

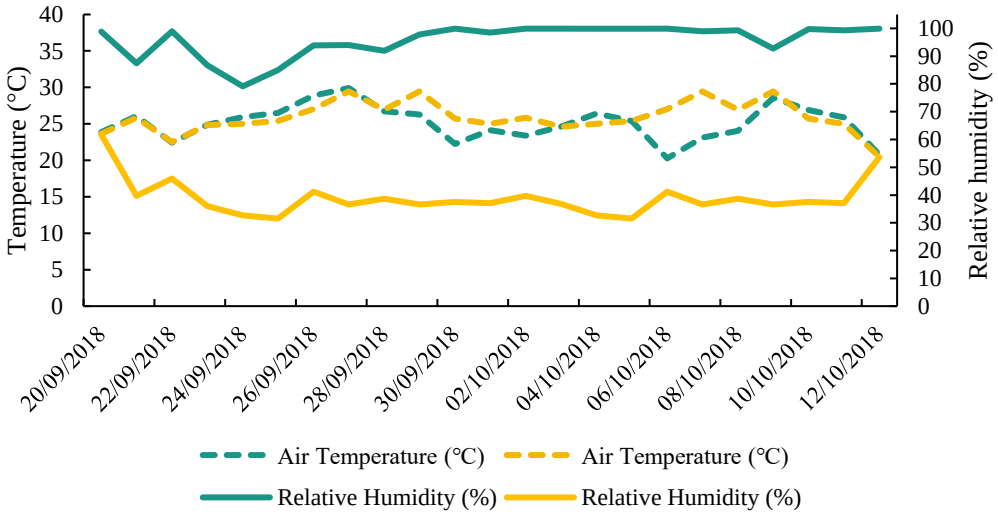


Figure 3. Daily averages of the growth conditions in the high humidity and low humidity chambers, throughout the experiment. Measurements recorded using a Fisher Scientific Traceable Humidity/Temperature. Dew-Point Meter (Fisher, UK). Green (■) lines represent recordings in the high humidity chamber, whilst yellow (■) lines represent recordings in the low humidity chamber.

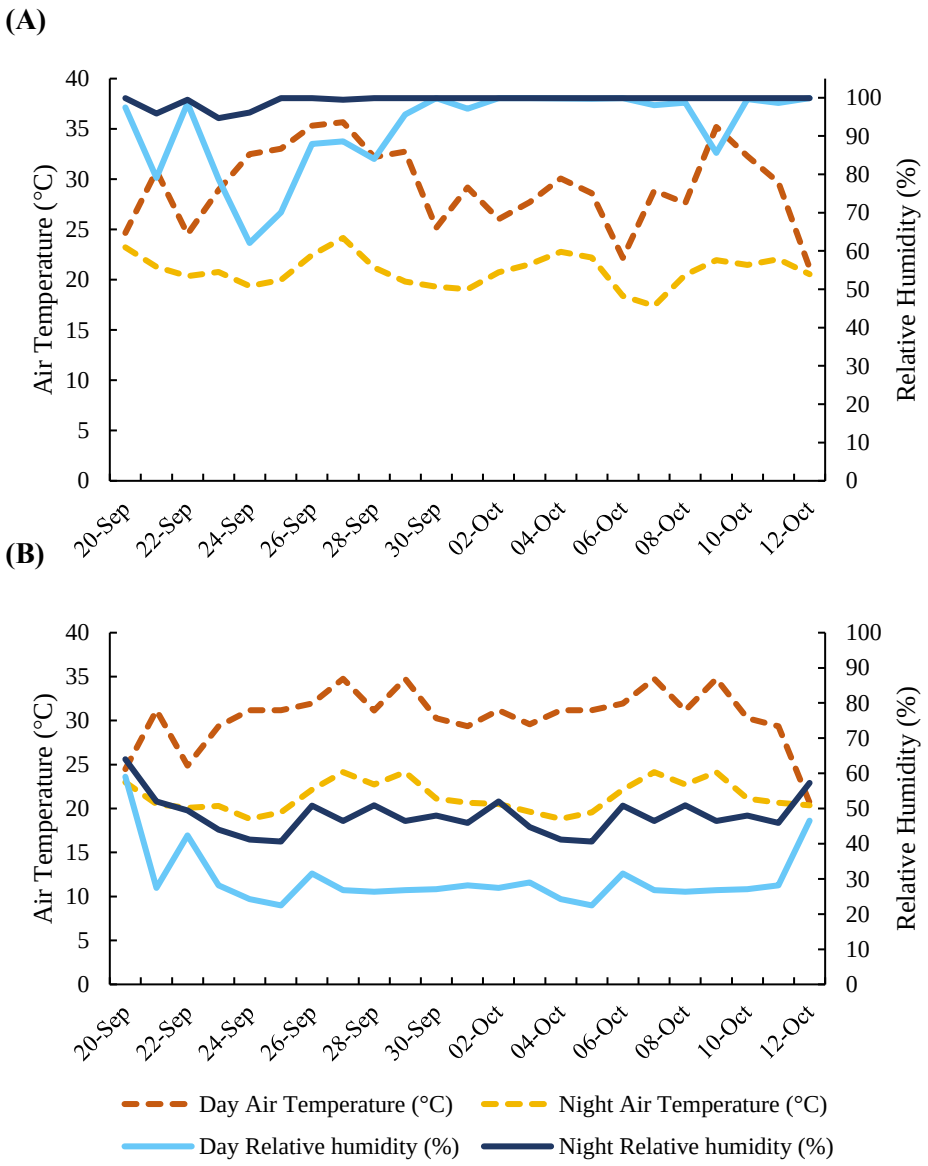


Figure 4. Day and night average temperature and relative humidity in the high humidity growth chamber (A) and the low humidity chamber (B). Measurements were recorded using a Fisher Scientific Traceable Humidity/Temperature. Dew-Point Meter (Fisher, UK). Day refers to 06:00 – 18:00 and night 18:01 – 05:59.

The number of biological reps was 3 for all species and treatments apart from maize LHHS whereby plant death rendered n=2.

2.3.2 Physiology Measurements

All measurements presented in this study were carried out on maize two weeks after germination, and wheat three weeks after germination.

2.3.2.1 Stomata

Stomatal impressions were carried out following the dental putty impression method described in (Coup*e et al*, 2006), using Affins Perfect Impressions, light body putty (Coltene/Whaledent AG, Switzerland). Stomatal impressions were taken from the middle third section on the two of the longest unfurled leaves per plant (leaves 3 and 4, so all leaves sampled were at the same developmental stage). Impressions were made on both the abaxial and adaxial sides of the leaf, three peels per side, per leaf with main veins avoided (six peels per leaf, 3 abaxial and 3 adaxial).

Stomatal counts were made directly through the field of view of a light microscope at ×25 magnification for maize and ×16 for wheat. One count was recorded for each peel, three peels per side of a leaf, two leaves sampled per rep, these were then averaged to give a biological rep. Maize n=3 apart from the LHHS treatment where n=2, wheat n=3 (all treatments).

Stomatal dimension measurements were made on images acquired on the light microscope at ×25 magnification for both maize and wheat. 30 stomata were measured per treatment, for both the abaxial and adaxial side of the leaf, which was then averaged to give the biological reps. Stomatal size (defined here as guard cell length multiplied by the total width of the guard cell pair as described in Franks & Beerling, 2009) (Figure 5) were measured in open source software Fiji

(Schindelin *et al.*, 2012) with the scale set from a graticule image. These observations on stomatal aperture recorded whether stomata were open or closed, all stomata reported as open were done so regardless of the degree of stomatal opening. Stomata were reported closed were completely shut, encompassed by two turgid guard cells.

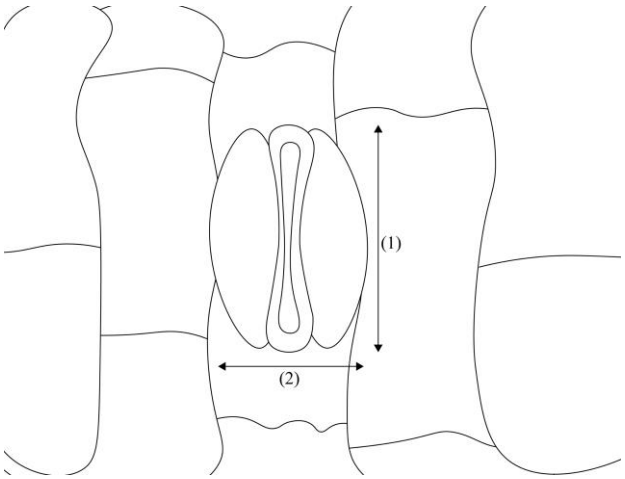


Figure 5. Schematic of a graminaceous stomatal pore (not to scale), displaying dimensions of interest when determining stomatal size. (1) Total pore length (2) Total pore width.

Maximum stomatal conductance (G_{max}) to both CO_2 and water vapour was calculated using the following equation from Franks and Beerlin (2009).

$$g_{w\max} = \frac{d}{v} \cdot D \cdot a_{\max} \Big/ \left(l + \frac{\pi}{2} \sqrt{a_{\max}/\pi} \right) \quad \text{(Equation 1)}$$

Where d is the diffusivity of water or CO_2 or in the air ($m^2\ s^{-1}$), v is the molar volume of air ($m^3\ mol^{-1}$) at 25°C, D is stomatal density (mm^2), a_{max} is the maximum area of the

stomatal pore (μm^2), l is the depth of stomatal pore (μm) and π is the mathematical constant equal to approximately 3.142.

As the g_{smax} of water and CO_2 is proportional and essentially comparable, only the g_{smax} of water values will be presented in the results.

2.3.2.2 Photosynthesis Measurements

To gain an understanding of photosynthesis responses to treatment conditions, a hand-held MultispeQ V2.0 device (Kuhlgert *et al.*, 2016) was used which consists of a leaf cuvette to record photosynthetic (chlorophyll fluorescence) parameters, ambient environmental conditions and Soil Plant Analysis Development (SPAD)-like measurements for relative chlorophyll content. Descriptions of recorded parameters are presented in Table 2. The data was then accessed via PhotosynQ, an open data platform (www.photosynq.org). Three measurements were taken from the longest and second-longest unfurled leaves (same leaves as stomatal impressions and same developmental stage), the measurements were taken from the base, middle and tip of the leaf. The device replicates external light intensity in the cuvette but has no other environmental regulation.

Table 2. Recorded MultispeQ V2 parameters. The device measures chlorophyll fluorescence and spectral reflectance to provide information on photosynthetic and environmental parameters as well as pigmentation information in terms of relative chlorophyll content.

Parameters discussed	**Description**
Quantum yield of photosystemII (ΦPSII)	The operational quantum efficiency of photosystemII in the light (Fm'-Fs') / Fm' (Murchie and Lawson, 2013).
ΦNPQ	The ratio of absorbed light energy 'partitioned' towards non-photochemical quenching (Murchie and Lawson, 2013).
Relative Chlorophyll Content	Measurement of relative chlorophyll content via spectral reflectance.
Leaf temperature differential	Leaf temperature minus ambient temperature.
Photosynthetically active radiation (PAR) µmol photons m^{-2} s^{-1}	The portion of the light spectrum utilised by plants (400-700nm) expressed as quantum (photon) flux.

2.3.2.3 Biomass Measurements

Fresh weights were recorded for the roots and shoots of both the maize and wheat, at time of harvest (maize two weeks of growth and wheat after three weeks). Leaf length measurements were carried out on the longest unfurled leaf of each plant (3 plants per treatment). Then the plant material was placed in the oven at 60 °C for 48 h. The material was weighed again, yielding dry mass values. For both fresh and dry weights, the roots and shoots of plants were weighed separately so the above and below-ground biomass values could be distinguished.

2.3.2.4 Root traits

Through the investigation of root biomass and root system architecture, we can see how the treatments are affecting the exploratory role of roots, which in turn affect water and nutrient acquisition. Above and belowground plant biomass measurements were made.

Roots were separated from shoots and gently washed straight after harvest. The whole root system was then laid out on black felt and photographed using a DSLR (Nikon 3200). The images were then imported and analysed in open source software Fiji (Schindelin *et al.*, 2012) using the plugin SmartRoot. This software enabled the measurement of root lengths, diameter, lateral root counts. For further information regarding the SmartRoot software see Lobet *et al*., (2011). Root trace images were produced with a line width of 5 and the scale bar representing 5 cm on each image.

2.3.3 Statistical Analysis

All statistical analyses were carried out in Genstat 20th Edition. Treatments and effects were compared using a general ANOVA with main effects of soil moisture

and humidity tested as well as any significant interaction between the two at the 5% level. When significance was detected, post hoc Tukey tests were carried out on balanced data and Fisher's unprotected least significant difference test on unbalanced data, when different letters are detected they are presented on figures. Chi-squared tests were carried out on the aperture data (open/closed) to test for a significant relationship between open and closed stomata and the treatment conditions.

2.4 RESULTS

2.4.1 Stomata

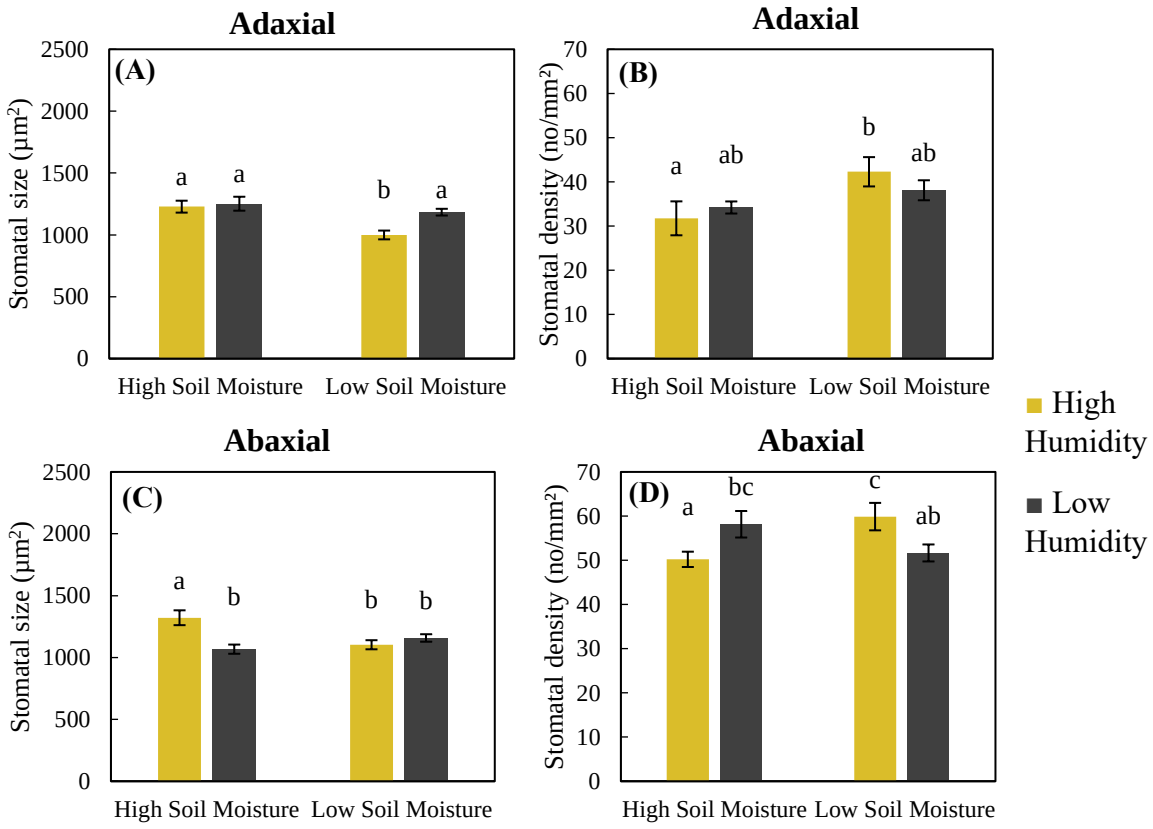


Figure 6. The effects of the four treatments: High Humidity High Soil moisture (HHHS), Low Humidity High Soil moisture (LHHS), High Humidity Low Soil Moisture (HHLS) and Low Humidity Low Soil moisture (LHLS) on stomatal size and density on maize plants two weeks after germination. Values presented show the differences between

abaxial and adaxial stomatal size (A and C) defined here as guard cell length multiplied by the total width of the guard cell pair. Also plotted is abaxial and adaxial stomatal density (B and D). Different letters represent significant difference at the 5% level after a post-hoc Fisher's unprotected least significant difference test. n=3 apart from LHHS where n=2.

Maize adaxial stomatal size (Figure 6A) was significantly affected by both soil moisture and humidity as main effects ($P = <0.001$ and $P=0.018$ respectively). During low soil moisture conditions, adaxial stomata were significantly smaller when humidity was high. Maize adaxial stomatal density (Figure 6B) was significantly affected by soil moisture as a main effect ($P=0.002$), with significantly higher stomatal densities under low soil moisture conditions. Whereas abaxial stomata (Figure 6C) were significantly affected by humidity as a main effect ($P=0.021$) and a significant interaction between humidity and soil moisture ($P<0.001$). During high soil moisture conditions, stomata were significantly larger when humidity is high. Maize abaxial stomatal density (Figure 6D) was significantly affected by an interaction between humidity and soil moisture ($P<0.001$) whereby under high soil moisture conditions, significantly higher densities were observed under low humidity conditions, in contrast, under low soil moisture conditions, significantly higher abaxial stomatal densities were observed. There were significant differences between treatments when looking at wheat abaxial and adaxial stomata separately (Figure 7).

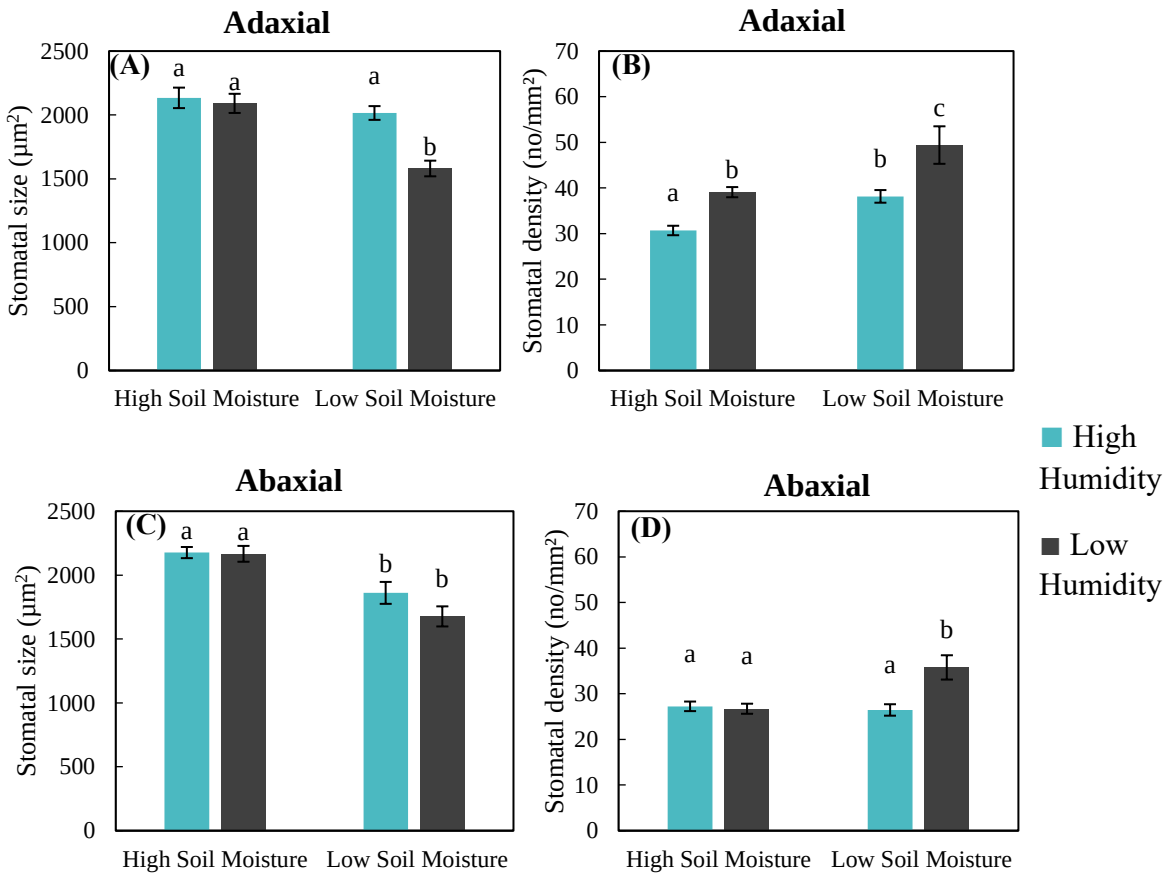


Figure 7. The effects of the four treatments: High Humidity High Soil moisture, Low Humidity High Soil moisture, High Humidity Low Soil Moisture and Low Humidity Low Soil moisture on stomatal size and density in wheat plants three weeks after germination. Values presented show the differences between abaxial and adaxial stomatal size (defined here as guard cell length multiplied by the total width of the guard cell pair) (A and C) as well as abaxial and adaxial stomatal density (B and D). Different letters represent significance at the 5% level after a post-hoc Tukey test. n=3.

Wheat adaxial stomatal size (Figure 7A) was significantly affected as humidity and soil moisture as main effects (each with $P<0.001$), as well as a significant interaction between humidity and soil moisture ($P=0.005$). Under low soil moisture conditions, adaxial stomata were significantly smaller when humidity was low. Adaxial stomatal densities (Figure 7B) were significantly lower under

high humidity conditions ($P<0.001$) and high soil moisture conditions ($P<0.001$). Abaxial stomata (Figure 7C) were significantly smaller in low soil moisture conditions ($P<0.001$). Whilst abaxial stomatal density (Figure 7D) was significantly affected by both humidity and soil moisture as main effects ($P=0.001$ and $P=0.002$ respectively), as well as a significant interaction ($P<0.001$). Under low soil moisture conditions, low humidity leads to significantly higher stomatal densities.

2.4.1.1 Stomatal aperture

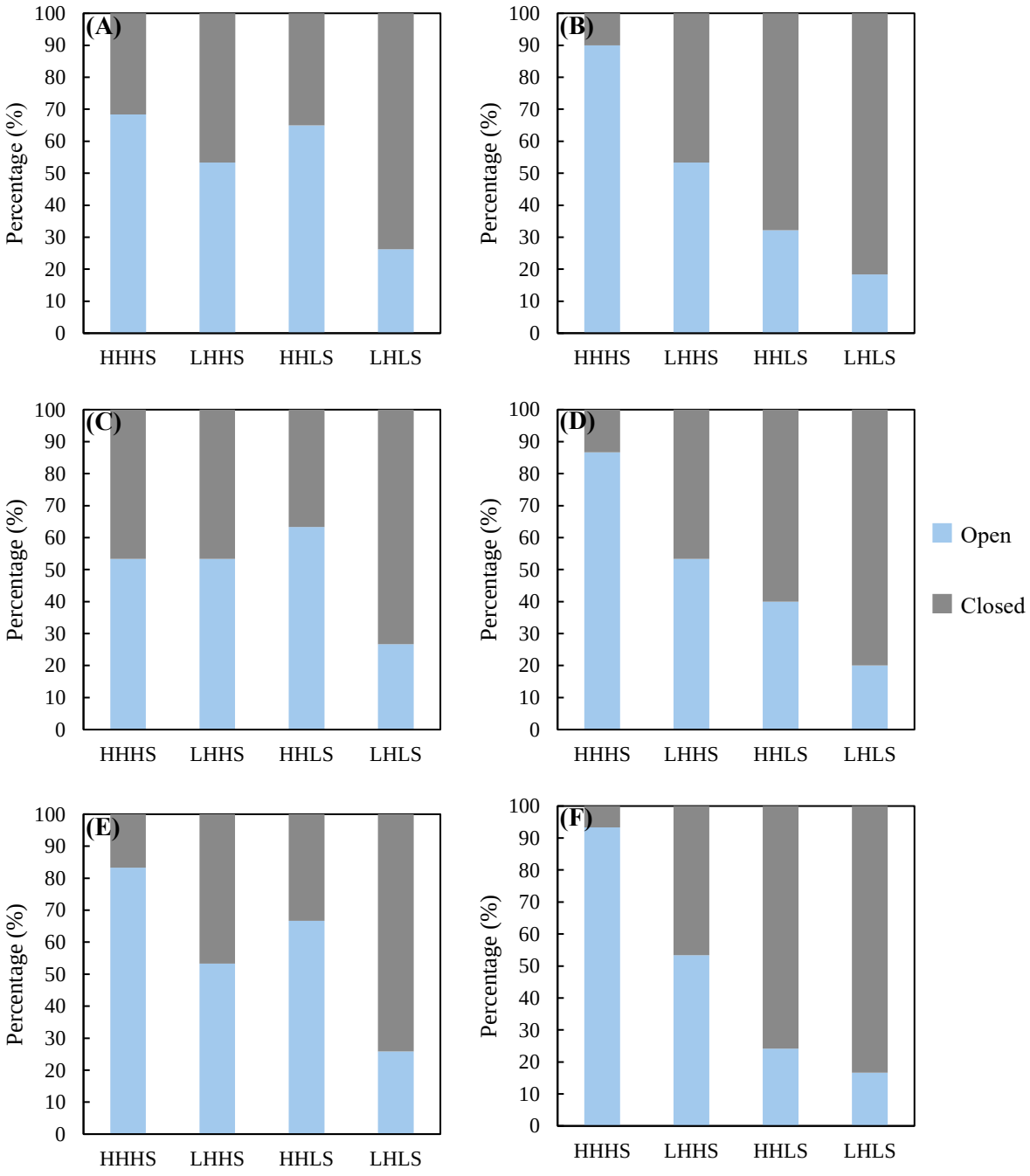


Figure 8. Stomatal aperture for maize whole leaf average (A), abaxial (C) and adaxial (E) sides of the leaf, maize plants two weeks after germination and wheat whole leaf average (B), abaxial (D) and adaxial (F), three weeks after germination and growth in the four treatment conditions: High Humidity High Soil moisture (HHHS), Low Humidity High Soil Moisture (LHHS), High Humidity Low Soil Moisture (HHLS) and Low Humidity

Low Soil Moisture (LHLS). Significant interaction of treatments on proportion of open/closed stomata are presented as a P-value after Chi-squared significance test at the 5% level, on count data. n=3 apart from LHHS where n=2 (Maize) n=3 (Wheat). Maize Chi2 = (A) P<0.001, (C) P=0.031, (E) P<0.001, Wheat = (B) P<0.001, (D) P<0.001, (F) P<0.001.

The treatment conditions significantly affect the proportion of open and closed wheat stomata on the whole leaf (Figure 8) (A), adaxial (B) and abaxial (C) surface (P<0.001, P<0.001 and P<0.001, respectively). Like maize, both sides of the leaf responded to the treatment conditions in a similar fashion.

Unlike maize, wheat (Figure 8) appeared more responsive to soil moisture conditions with most stomata closed when soil moisture was low, high humidity did not lead to more stomata opening when soil moisture was low. Low humidity also led to increased stomatal closure in wheat.

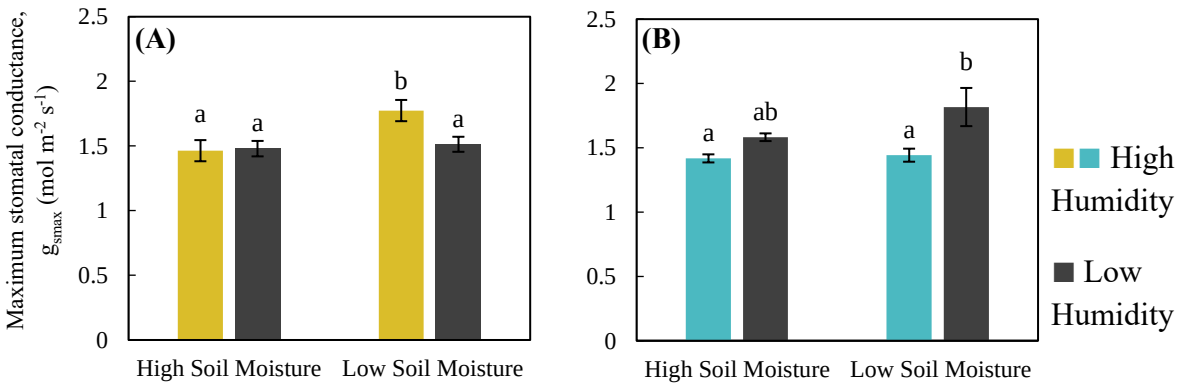


Figure 9. Two panels displaying the global maximum stomatal conductance (g_{smax}) calculated for maize and wheat, to water vapour across the four treatments: High Humidity High Soil moisture, Low Humidity High Soil moisture, High Humidity Low

Soil moisture and Low Humidity Low Soil moisture. Means of predicted g_{smax} of water vapour in maize (A) and wheat (B) are presented with error bars indicating ±SE. Different letters represent significance at the 5% level after a post-hoc Fisher's unprotected least significant difference test. n=3 apart from LHHS where n=2 for maize and post-hoc Tukey test for wheat (n=3).

Maize maximum stomatal conductance (g_{smax}) to water vapour (Figure 9A), (calculated as in Franks and Beerling (2009)), was significantly affected by humidity and soil moisture as main effects (P=0.03 and P=0.003 respectively, as well as a significant interaction between the two (P=0.015), under low soil moisture conditions, g_{smax} of water vapour was significantly higher during high humidity conditions.

Similarly in wheat, there were significant main effects of humidity ($P < 0.001$) and soil moisture (P=0.043) on g_{smax} of water vapour (Figure 9C) however, humidity had the opposite effect under low soil moisture conditions, with high humidity resulting in significantly lower g_{smax} values.

2.4.2 Photosynthetic Parameters

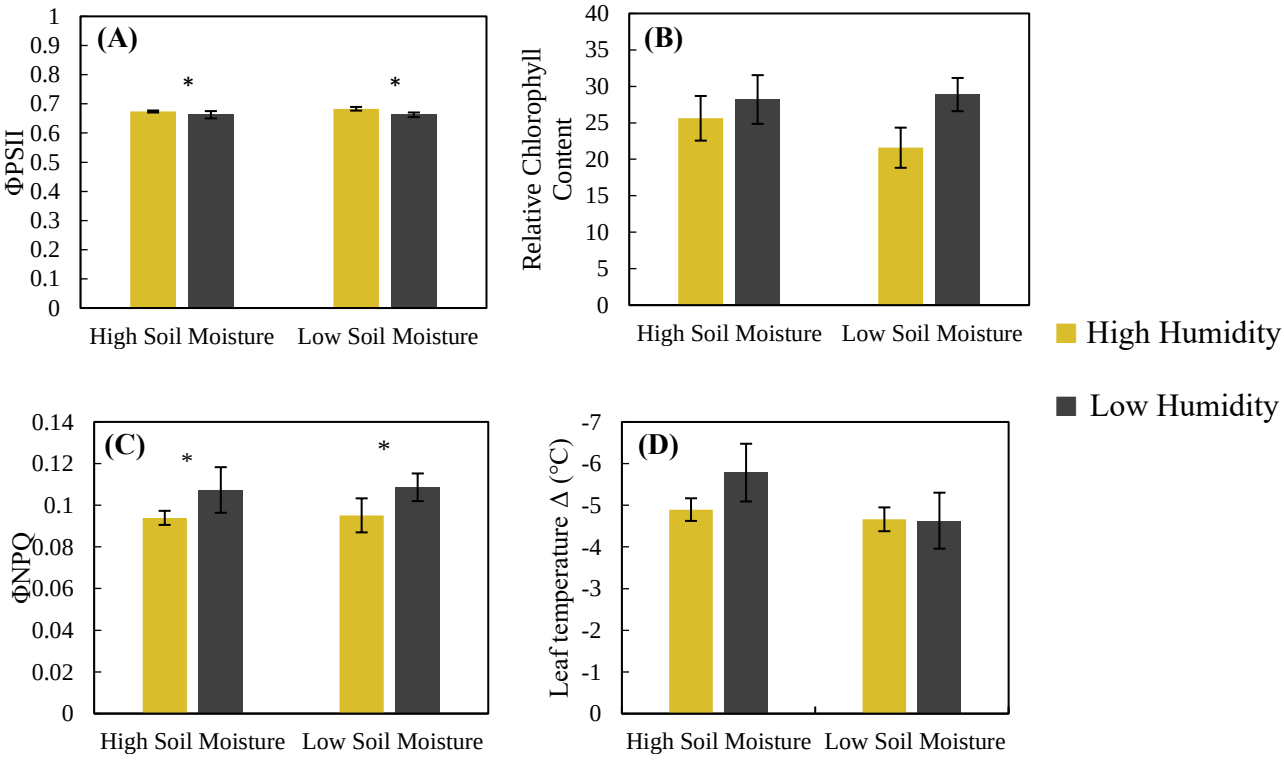


Figure 10. The effects of the four treatments High Humidity High Soil moisture (HHHS), Low Humidity High Soil moisture (LHHS), High Humidity Low Soil moisture (HHLS) and Low Humidity Low Soil moisture (LHLS) on maize plants two weeks after germination. Data collected from the multispec Photosynq with measurements of (A) ΦPSII, (B) relative chlorophyll content, (C) ΦNPQ, and (D) leaf temperature differential are presented with error bars displaying ± SE values. Significance of main effects, from General ANOVA, are represented by * ($P<0.05$). n =3 apart from LHHS where n=2.

ΦPSII, the quantum yield of photosystem II (Figure 10A) of maize was significantly higher in plants grown under high humidity conditions ($P=0.04$), irrespective of soil moisture content although differences were small in magnitude. ΦPSII at the same light intensity is an accurate measurement of the rate of electron flow through PSII and is notable here that there are minimal differences. This does not necessarily provide information on rates of

photosynthesis however ΦNPQ in maize (Figure 10C) was significantly lower under high humidity conditions (P=0.04) indicating less energy used in photoprotective processes. Relative chlorophyll content (Figure 10B), and leaf temperature differential (Figure 10D) were not significantly affected by the treatment conditions at the 5% level.

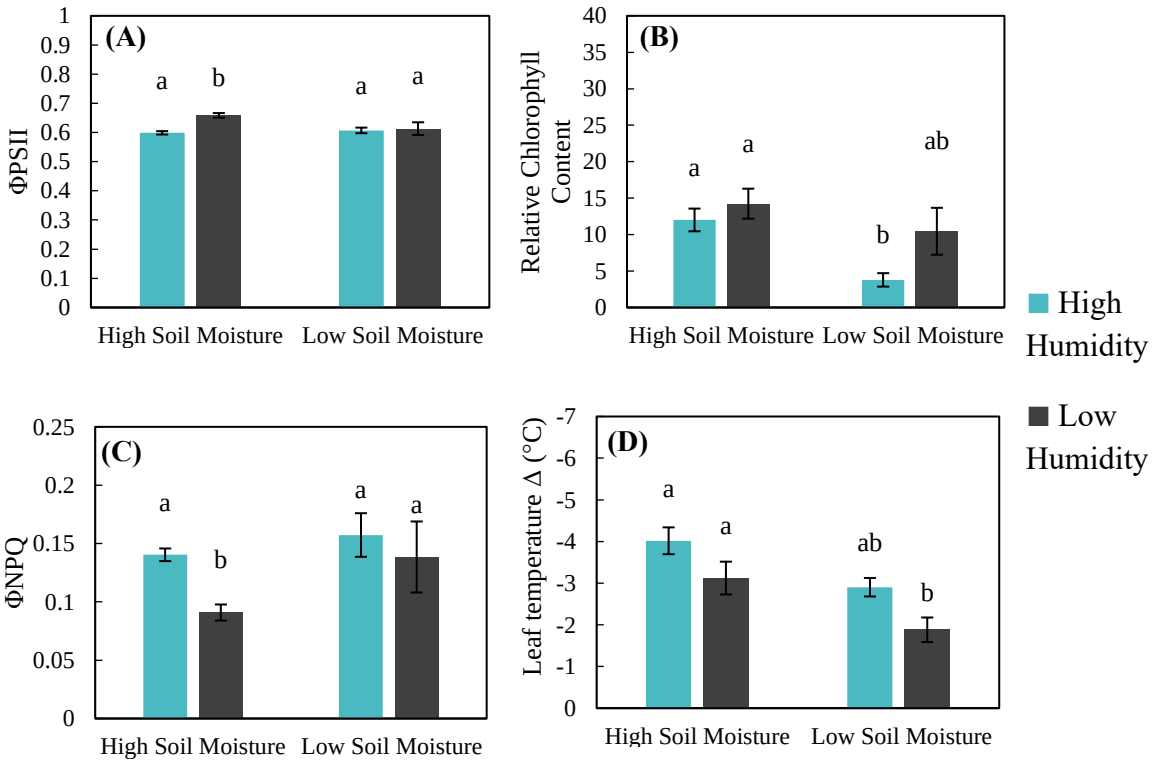


Figure 11. The effects of the four treatments High Humidity High Soil moisture, Low Humidity High Soil moisture, High Humidity Low Soil moisture and Low Humidity Low Soil moisture on wheat three weeks after germination. Data collected from the multispec Photosynq with measurements of (A) ΦPSII, (B) relative chlorophyll content, (C) ΦNPQ, and (D) leaf temperature differential between leaf and atmosphere, are presented with error bars displaying ± SE values. Different letters represent significance at the 5% level after a post-hoc Tukey test. n=3.

Wheat photosynthetic responses differed from maize. Unlike maize, wheat ΦPSII (the quantum yield of photosystem II) (Figure 11A) was significantly affected by

both soil moisture and humidity as the main effect (P=0.049, P=0.002 respectively), as well as a significant interaction between soil moisture and humidity (P=0.048). During high soil moisture conditions, high humidity resulted in significantly lower ΦPSII values. ΦNPQ (Figure 11C) was significantly affected by both soil moisture and humidity as the main effect (P=0.029 and P=0.018 respectively). Under low humidity conditions, high humidity resulted in significantly higher ΦNPQ values. Wheat relative chlorophyll content (Figure 11B) was affected by soil moisture as the main effect, with low soil moisture resulting in lower chlorophyll content, regardless of humidity conditions. Unlike maize, wheat leaf temperature differential (Figure 11D) was significantly affected by both soil moisture and humidity as main effects (P=0.005 and P=0.025 respectively), with both low soil moisture and low humidity resulting in warmer leaves.

2.4.3 Biomass

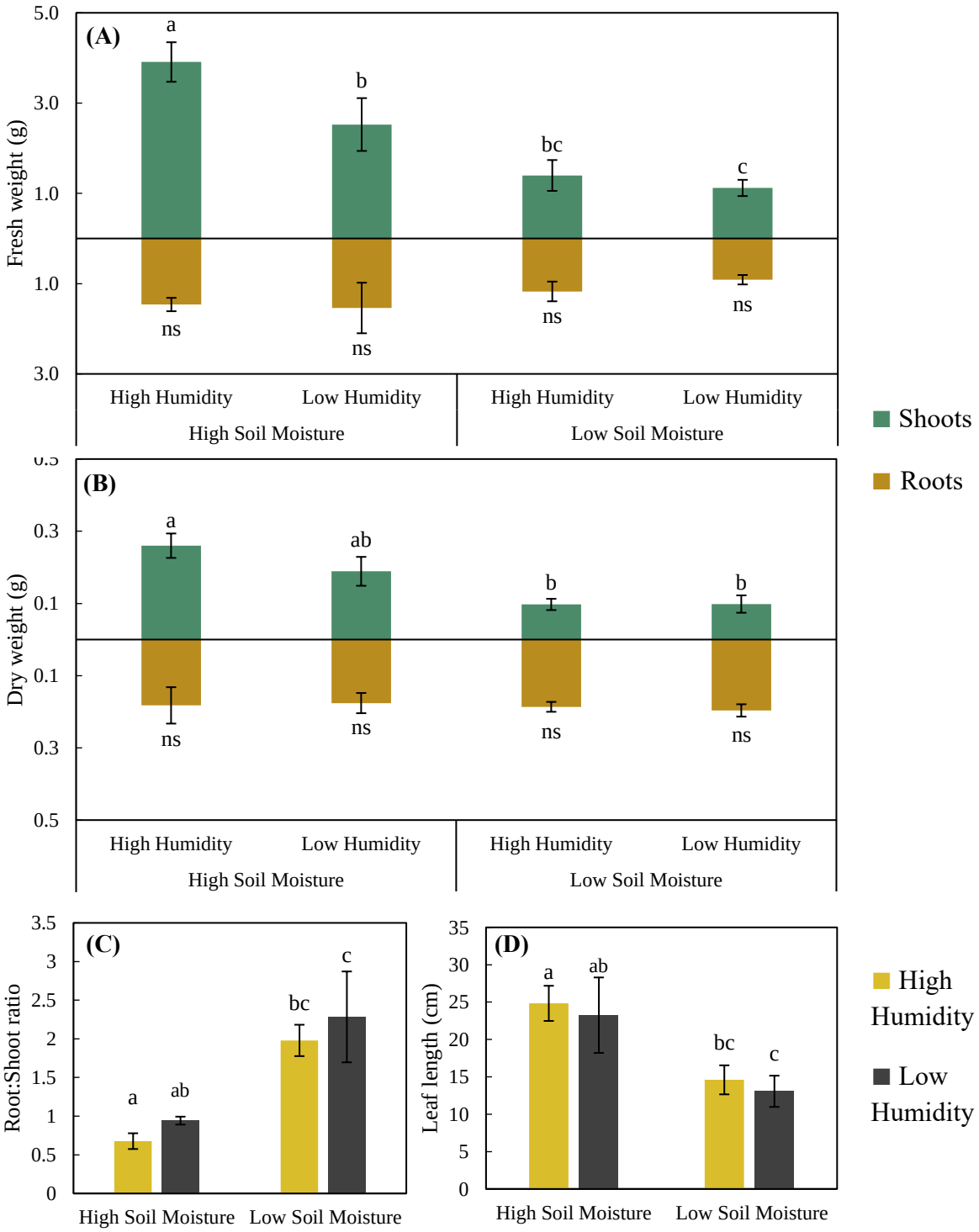


Figure 12. Maize shoot and root fresh weights (A), dry weights (B), the root:shoot ratio (dry weight) (C) and the length of the longest unfurled leaf (panel D), in response to treatment conditions High Humidity High Soil moisture (HHHS), Low Humidity High

Soil moisture (LHHS), High Humidity Low Soil moisture (HHLS) and Low Humidity Low Soil moisture (LHLS), two weeks after germination. Means are presented with error bars representing ± SE. Different letters present significance at the 5% level after a post-hoc Fisher's unprotected least significant difference test. ns represents no significant difference. n=3 apart from LHHS where n=2.

Maize shoot fresh weight was significantly higher in high humidity (P=0.037) and high soil moisture (P=0.001) (Figure 12A), with the highest maize shoot fresh weight in the HHHS treatment. Shoot dry weight (Figure 12B), was significantly higher in high soil moisture conditions regardless of humidity (P=0.002). Echoing root fresh weight results, there were no significant treatment effects on root fresh dry weights at the 5% level. However, the maize dry weight root:shoot ratio (Figure 12C) was significantly higher in low soil moisture conditions (P=0.008). Leaves were also significantly shorter in low soil moisture conditions (P=0.006) (Figure 12D).

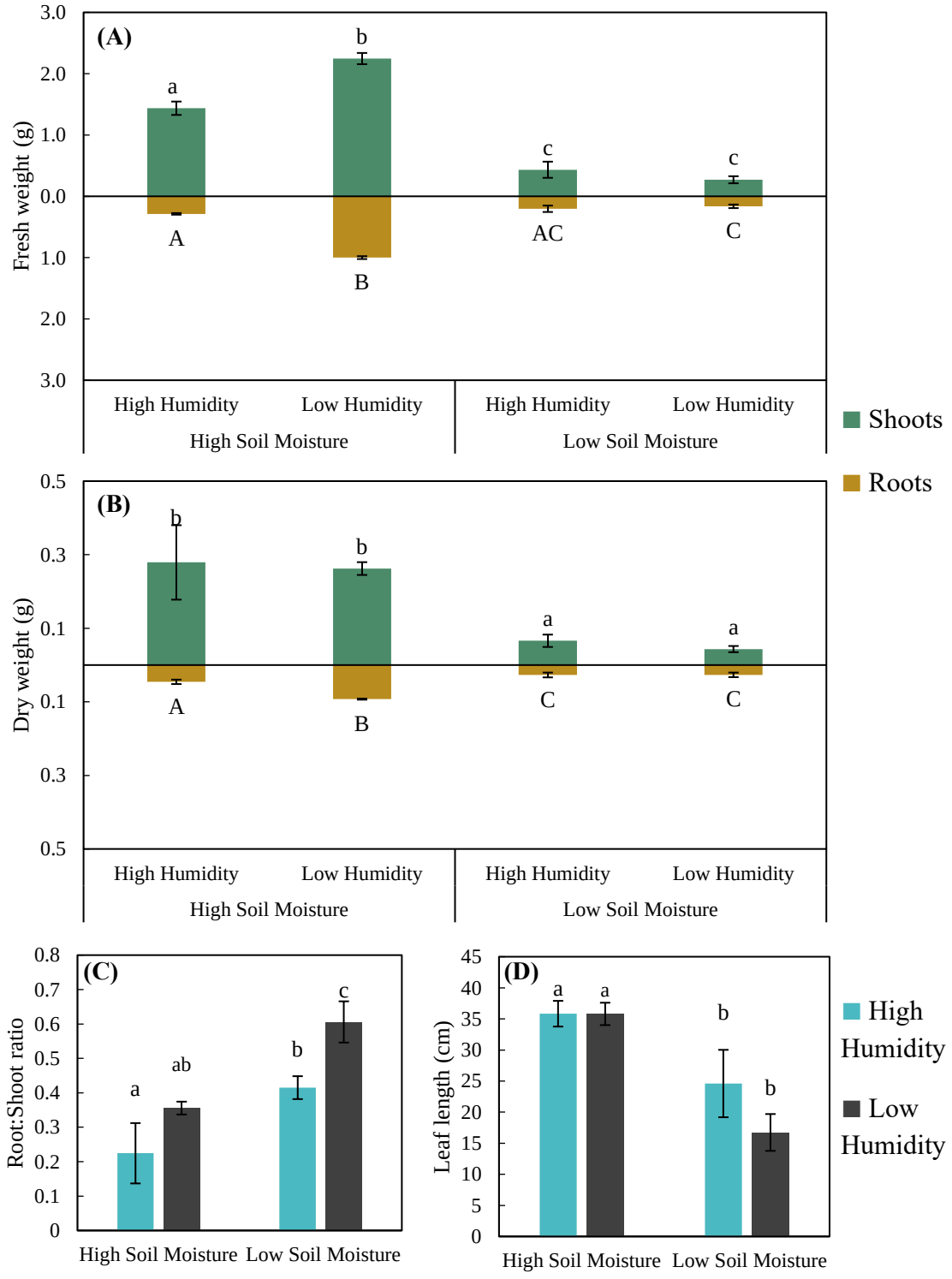


Figure 13. Wheat shoot and root fresh weights (A), dry weights (B), the root:shoot (dry weight) ratio (C) and the length of the longest unfurled leaf (D), in response to treatment conditions High Humidity High Soil moisture, Low Humidity High Soil moisture, High Humidity Low Soil moisture and Low Humidity Low Soil moisture, three weeks after

germination. Means are presented with error bars representing ± SE. Different letters represent significance at the 5% level after a post-hoc Tukey test. n=3. With regards to panels A and B, upper case letters represent significance between treatments in the shoots, whilst lower case letters represent a significant difference between treatments in the roots.

Wheat biomass was more sensitive to soil moisture than maize. Wheat shoot fresh weight (Figure 13A) was significantly affected by soil moisture and humidity as main effects ($P<0.001$ and $P=0.013$ respectively), as well as a significant interaction between soil moisture and humidity ($P=0.001$). High soil moisture resulted in significantly higher shoot fresh weights, and within the high soil moisture conditions, low humidity led to significantly higher shoot fresh weights with the highest average shoot fresh weight values found in the LHHS treatment. Although no significant treatment effects were found on the fresh weights of maize roots, in wheat, however, root fresh weight (Figure 13A) was significantly affected by humidity and soil moisture as main effects (both with $P<0.001$), as well as a significant interaction between humidity and soil moisture ($P<0.001$). Root fresh weight was higher in high soil moisture conditions and further increased by low humidity.

Similar to maize, wheat shoot dry weight (Figure 13B) was significantly higher in high soil moisture conditions($P=0.003$). Unlike maize, wheat root dry weight was significantly affected by both soil moisture and humidity as main effects ($P<0.001$ and $P=0.003$ respectively), and a significant interaction between soil moisture and humidity ($P=0.002$). Echoing root fresh weight findings, high soil moisture led to

significantly higher root dry weight, which was further increased under low humidity conditions.

The wheat dry weight root:shoot ratio (Figure 13C) was significantly higher in low soil moisture conditions (P=0.005) echoing maize. However, the wheat root:shoot ratio was also significantly affected by humidity (P=0.021) with low humidity resulting in higher root:shoot ratios. Comparable to maize, wheat leaf length (Figure 13D), was significantly shorter under low soil moisture conditions (P=0.002).

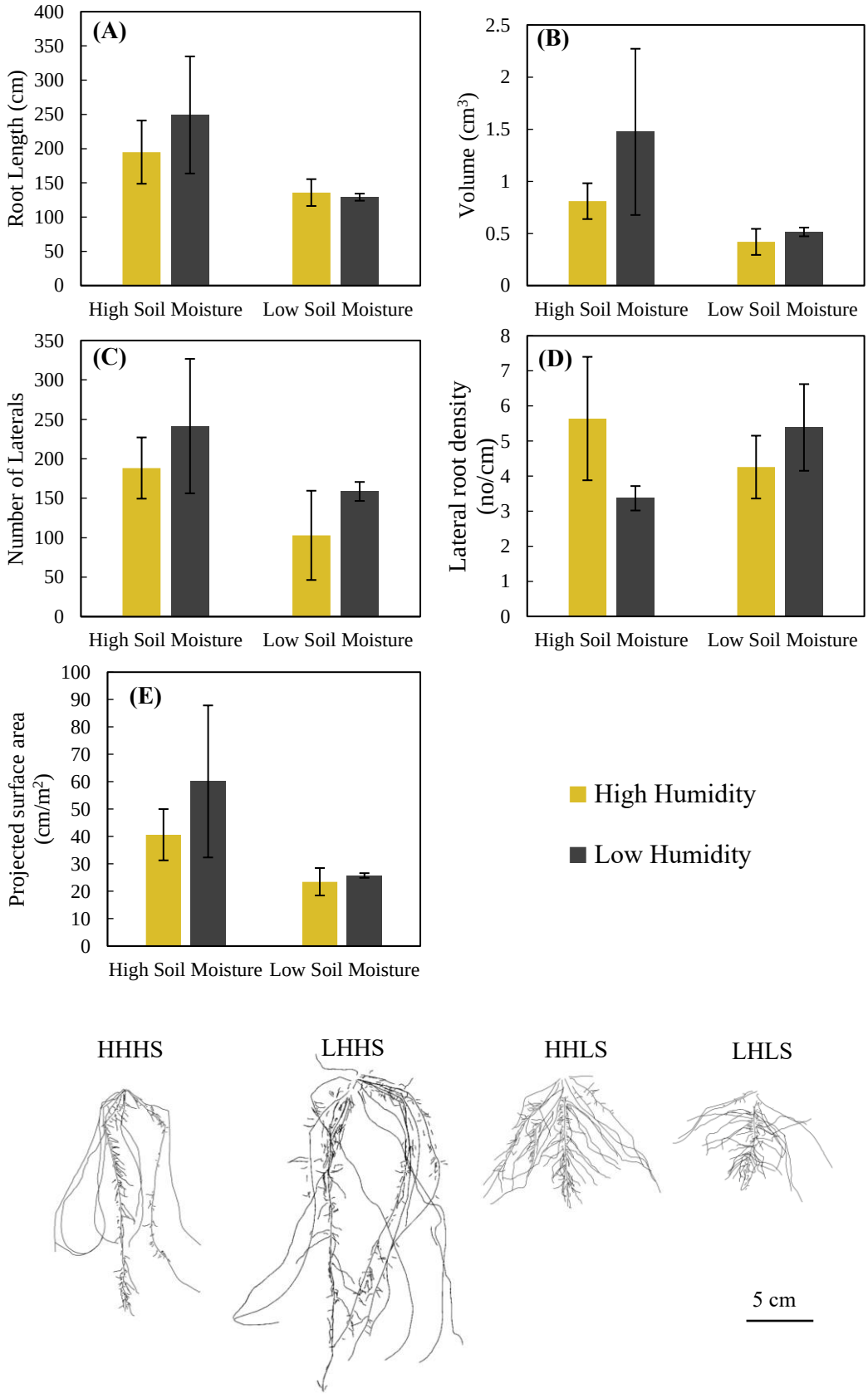


Figure 14. Root trace data on total root system length (A), total root system volume (B), the average number of laterals (C), lateral root density (D), and projected surface area (E)

in response to treatment conditions: High Humidity High Soil moisture (HHHS), High Humidity Low Soil moisture (HHLS), Low Humidity High Soil moisture (LHHS) and Low Humidity Low Soil moisture (LHLS), measurements made on maize two weeks post-germination. Values presented are mean values ± SE. n=3 apart from LHHS where n=2.

There were no significant treatment effects on maize root morphology (Figure 14).

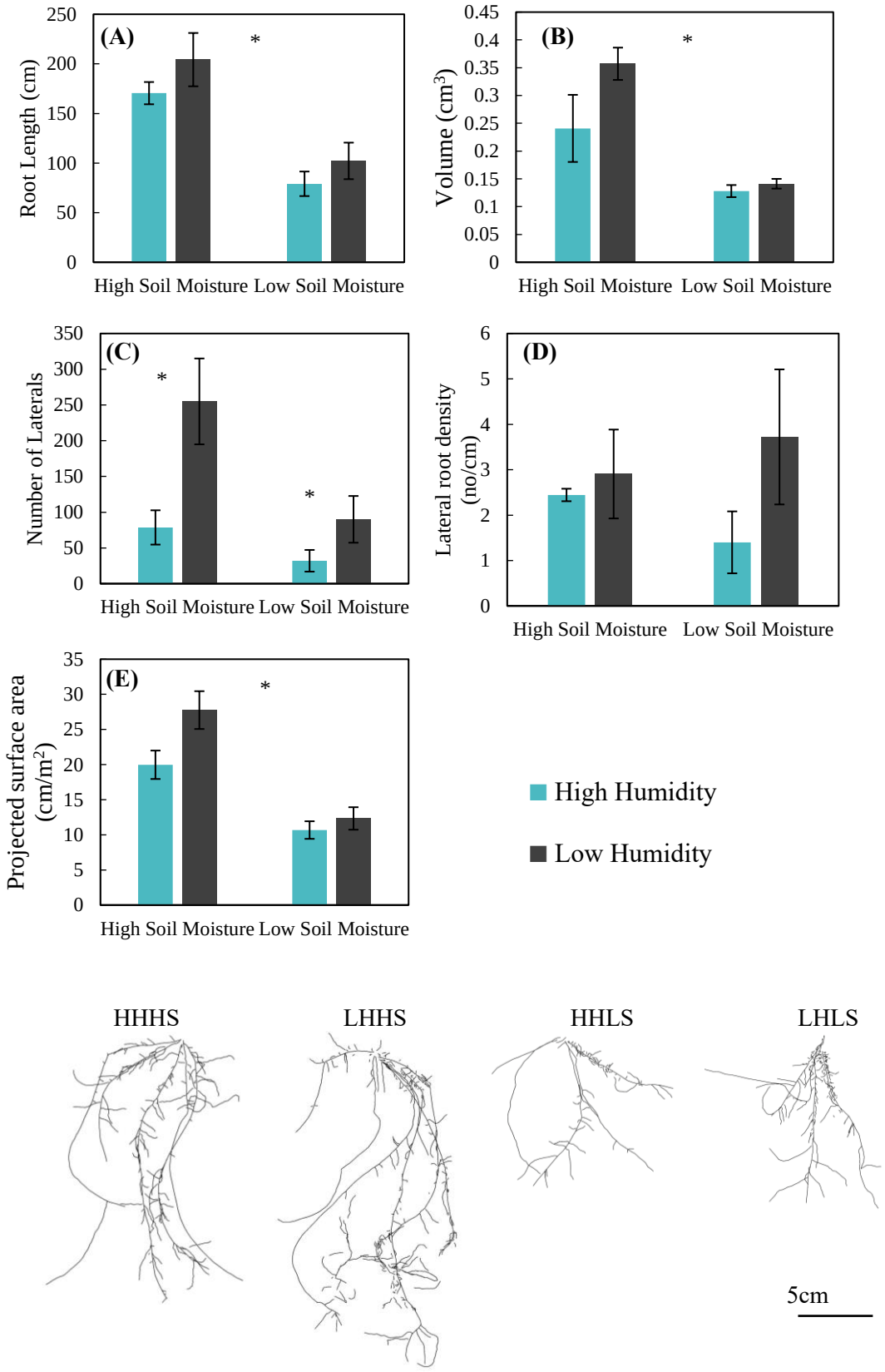


Figure 15. Root trace data on total root system length (A), total root system volume (B), the average number of laterals (C), lateral root density (D), and projected surface area (E).

in response to treatment conditions High Humidity High Soil moisture (HHHS), High Humidity Low Soil moisture (HHLS), Low Humidity High Soil moisture (LHHS) and Low Humidity Low Soil moisture (LHLS), in wheat three weeks post-germination. Values presented are mean values ± SE. * represents significance at the 5% level of main effects after a general ANOVA n=3.

Low soil moisture resulted in significantly reduced wheat root lengths (P=0.003), volume (0.004) and projected surface area (Figure 15). Whereas low humidity resulted in a significantly higher number of lateral roots (Figure 15C)

2.5 DISCUSSION

Plant responses to reduced soil moisture content are widely explored. In this chapter, we focus on de-coupling the responses that are specific to soil moisture with those that result from altered atmospheric humidity.

Overall, in maize, high humidity led to slightly higher ΦPSII and lower ΦNPQ values, with a majority of stomata remaining open despite low soil moisture conditions, thus implying high humidity is increasing the plants photosynthetic capacity potentially creating a more productive plant with higher biomass. However, in wheat, it is quite the opposite. High humidity conditions witnessed high ΦNPQ values, with a majority of stomata closed during low soil moisture conditions. ΦPSII was highest under LHHS conditions suggesting that soil water is a greater influence on the photosynthetic capacity of wheat compared to humidity. The discussion will explore this over-arching hypothesis

2.5.1 Maize

2.5.1.1 Stomatal Morphology

The effect of humidity on maize stomatal morphology (size and density) (Figure 6) as dependent on the soil moisture conditions in which the plants were grown. When soil moisture was high, the high humidity treatment resulted in larger and less dense stomatal arrangements, whereas when soil moisture was low, high humidity drove smaller and more frequent stomata (Figure 6). This inverse relationship between stomatal size and density (Franks and Beerling, 2009; Bertolino, Caine and Gray, 2019) is therefore maintained in both soil moisture

regimes, regulated by humidity. This inverse relationship is thought to be due to larger stomata take up more room due to cell enlargement (Nejad and Van Meeteren, 2005), thus leaving less room for other stomata hence a decrease in density across the leaf surface or vice versa. Furthermore, during HHHS (high soil moisture high humidity) treatment conditions, the maize plants boasted some of the largest unfurled leaves (Figure 12), whereby the decreased stomatal density could have been driven by stomata spreading across the larger leaf surface due to epidermal cell expansion (Nejad and Van Meeteren, 2005). This could have been accounted for by looking at stomatal index which is the ratio of the number of stomata in a given area divided by the number of stomata and epidermal cells in the same area.

In the low soil moisture treatment, at high humidity (HHLS), though the leaves were large and comparable to the sizes in HHHS, stomata were smaller and more densely arranged. Suggesting that this stomatal adaptation was not a direct effect of leaf size. The smaller stomata and increased densities when grown in high humidity but low soil moisture (HHLS) could be a result of reduced turgor in the leaf driven by low soil moisture conditions and subsequent reduced soil water potential (Rodriguez-Dominguez *et al.*, 2016), such processes associated with leaf dehydration (Kim *et al.*, 2018). Reduced leaf turgor will lead to smaller cells which subsequent 'shrinkage' draws cells closer together, increasing cell density. Since stomatal density is inevitably affected by the cell density on the leaf surface (Wang, Chen and Xiang, 2007), any increase in leaf cell density will result in a subsequent increase in stomatal density. This could be the reason for increased stomatal densities in any of the treatments which could drive a loss in turgor (e.g.

low soil moisture, low humidity high VPD), resulting in reduced cell sizes and increased density. As such the smaller, densely arranged stomata are considered to exhibit greater gas conductance due to the shorter diffusion distances (Raven, 2014), and therefore associated with higher maximum theoretical stomatal conductance (g_{smax}) (Lammertsma *et al.*, 2011) this is reflected in Figure 9 whereby the g_{smax} was highest in the HHLS treatment.

Stomatal size and density play an important role in establishing water use efficiency across a range of environmental variables as demonstrated by recent work which has manipulated density in a range of species (Franks *et al.*, 2015; Bertolino, Caine and Gray, 2019; Dunn *et al.*, 2019; Mohammed *et al.*, 2019)**.** However, when considering the potential effects of soil moisture and humidity on stomatal morphology and gas exchange, we need to also consider the impact on stomatal aperture (whether the stomata are open or closed) (**Error! Reference source not found.**). Changing the number or density of stomata in response to environmental conditions will not make any difference if no stomata are open. Maize stomatal aperture showed some sensitivity to changes in humidity and soil moisture conditions, as there was only a greater proportion of closed stomata in the LHLS treatment (**Error! Reference source not found.**). In maize, a greater proportion of stomata are open during high humidity conditions, regardless of soil moisture content, suggesting that humidity also plays a large role in stomatal opening/closing in maize. However, there was an interesting humidity effect that is dependent on soil moisture conditions. When soil moisture was low, high humidity resulted in a greater proportion of stomata remaining open, comparable to apertures of stomata in high soil moisture conditions. This supports the notion

that stomata can directly respond to changes in VPD (Lange *et al.*, 1971; Holbrook *et al.*, 2002), remaining open despite roots experiencing low soil moisture conditions. This could be evidence that high humidity (low VPD) and subsequent reduced transpirational demand, reduce the stress placed on limited soil water reserves, resulting in less drought-stressed plants which have more stomata remaining open (Kübarsepp *et al.*, 2019). Also, maize plants require maintenance of midday leaf water potentials during transpiration (Carins Murphy, Jordan and Brodribb, 2014), therefore stomata open to increase the number of pores carrying out transpiration processes despite low VPD, thus maintaining xylem integrity and water status of plant tissues (Prieto, Lebon and Ojeda, 2010). However, regardless of the higher frequency of open stomata during high humidity conditions, and the calculated highest g_{smax} in the HHLS treatment (Figure 9), maize photosynthetic parameters (Figure 10) showed only small changes in response to humidity. Thus suggesting water taken up by the plant remains in the leaf tissue, and is not lost via g_{smax} during these conditions.

2.5.1.2 Photosynthetic Capacity

High humidity caused a very small increase in ΦPSII and a subsequent very small decrease in ΦNPQ (Figure 10). This very small treatment effect on photosynthetic parameters is not too surprising, considering the C4 nature of maize. C4 photosynthesis is less sensitive to perturbations in environmental conditions, stomatal morphology and subsequent stomatal conductance (Collatz, Ribas-Carbo and Berry, 1992). As stomatal conductance tends to be lower, and photosynthetic capacity higher, C4 plants boast higher WUE and greater drought tolerance. However, in this chapter we did observe that in maize, high humidity supports slightly higher ΦPSII and slightly lower ΦNPQ values (Figure 10), implying that

high humidity is increasing the efficiency of the initial light energy capture of the PSII reaction centre (Zhang *et al*., 2020), supported by the literature whereby higher rates of photosynthesis are found in high humidity and lower rates in low humidity (Rawson, Begg and Woodward, 1977; Bunce, 1984; Marsden, Lieffers and Zwiazek, 1996; Tanaka *et al.*, 2013; Du *et al.*, 2019). Nevertheless, in this chapter, the difference in ΦPSII between high and low humidity treatments was very small and without infrared gas analyser (IRGA) measurements, we cannot conclude that more carbon is gained due to higher rates of photosynthesis in high humidity. Furthermore, any potential increases in photosynthesis in high humidity would only be slight, and not substantial enough to affect plant productivity, as there were no recorded increases in maize root or shoot dry weight in response to humidity within this chapter (Figure 12).

2.5.1.3 Biomass

Though maize biomass production was not affected greatly by humidity, low soil moisture conditions resulted in significantly reduced shoot growth, with roots unaffected (Figure 12). In this chapter, maize grown in low soil moisture conditions were smaller, have shorter leaves and had a higher root:shoot (dry weight) ratio, echoing findings from previous studies (Sharp and Davies, 1979; Vadez *et al.*, 2007; Ruttanaprasert *et al.*, 2015; Studer, Hu and Schmidhalter, 2017; Ledo *et al.*, 2018). Biomass accumulation is intrinsically linked to stomatal conductance and the trade-off between carbon gained and water lost (Liu *et al.*, 2011). The reduced shoot dry weight in low soil moisture conditions is therefore likely to be due to restricted transpiration rates to mitigate water loss, reducing nutrient uptake from the roots as well a transport of nutrients to the shoots (Kramer, 1983) also leading to less carbon available for biomass production.

Moreover, dry soil conditions reduce the diffusion rates of nutrients across the soil matrix to the plant (Pessarakli, 1999) resulting in fewer nutrients reaching the plant hence reduced growth and biomass production (Hu, Burucs and Schmidhalter, 2008).

The biomass data can be explored further when looking at the dry weight root:shoot ratio (Figure 12), which can give us an indication on where maize favours resource use whether it be in light interception (shoots) or nutrient and water foraging (roots). In this chapter, during low soil moisture conditions, maize appears to sacrifice shoot biomass in favour of root growth, as presented in the increased root:shoot (Figure 12). Such an increase in root:shoot ratio is a trait commonly associated with drought tolerance (Eghball and Maranville, 1993; Karcher *et al.*, 2008) whereby plants invest more energy and resources into root growth (Studer, Hu and Schmidhalter, 2017), to facilitate soil exploration in search of water (Agathokleous *et al.*, 2018), as the procurement of soil resources is considered a fundamental limitation to crop production (Lynch, 2013). Therefore, supporting claims that the acquisition of soil resources is more important than canopy architecture and subsequent capture of light when plants are growing in sub-optimal conditions (Hammer *et al.*, 2009).

Interestingly, the root trace data (Figure 14) whereby a more in-depth analysis of the treatment effects on root growth was carried out, shows maize roots were significantly shorter in low soil moisture conditions echoing findings from Eghball and Maranville (1993). These results were somewhat unexpected as low soil moisture is considered to result in increased rooting depths to access deeper

water reserves (Henry *et al.*, 2012; Lynch, 2013). Though it is not unheard of for plants to adopt a severely hindered root growth strategy during resource (water and/or nutrients) deficit conditions, through a shallower and less dense root growth (Rich and Watt, 2013). However, maize root dry weight does not differ, which implies the shallower root response is not driven by metabolic costs reducing root density and mass, but rather an external factor driving changes to root length. Due to the experimental design, whereby maize plants were grown in columns and watering by weight was carried out to achieve treatment conditions, the water was applied to the soil surface as a top-down approach rather than trays. This could explain why there is heavy shallow branching in the LHLS treatment in maize, as the roots are exploring the upper soil surface where water reserves are more likely. Thus responding similarly to plants that are relying on rainfall irrigation during drought months, whereby shallower root systems are produced (Henry *et al.*, 2011). Root foraging is not just influenced by water but also macro and micronutrients in the soil. As such, nutrient location in the soil profile can influence rooting depths, with immobile nutrients (Bakker, 1991) for example, phosphorous, commonly concentrated in the top layers and causing greater topsoil foraging of phosphorous deficient plants. Furthermore, the shallower root system could also be caused by increase root penetration resistance, a trait often associated with drying soils (Bengough, Croser and Pritchard, 1997; Cairns *et al.*, 2011). Moreover, the top-down watering technique could be increasing the soil bulk density, compacting subsoil layers, further restricting root penetration and root access to water and nutrients in subsoil layers (Mu *et al.*, 2016).

During dry soil conditions, vertical root growth can be impeded therefore leading to the production of shallower root systems (Cairns *et al.*, 2011). During this chapter the soil was packed into the columns at a bulk density of 1.3g cm^{-3} which is leaning towards 'moderate' soil compaction (Grzesiak *et al.*, 2013), therefore any further increases in the bulk density could have serious consequences. Such mechanical impedance to root growth can have a particularly large impact on monocots such as maize and wheat, due to the high volume of adventitious roots initiating close to the soil surface, at the stem. Each of these new roots has to penetrate through the whole soil profile, and whilst in general, compaction has been found not to affect the number of adventitious roots, it does decrease their length (Grzesiak *et al.*, 2013), thus leading to shallower RSA (Colombi *et al.*, 2018).

2.5.2 Wheat

Compared to maize, wheat appeared more sensitive to changes in soil moisture as a main effect, as well as humidity effects that were soil moisture dependent.

2.5.2.1 Stomatal Morphology

Changes to wheat stomatal morphology (size and density) (Figure 7) in response to humidity are dependent on the soil moisture conditions.

When soil moisture is high, there are no obvious effects of humidity on wheat stomatal morphology. However, when grown under low soil moisture conditions high humidity results in larger and less dense stomata in the HHLS treatment (opposite results to maize whereby stomata were smaller and denser) compared to the LHLS treatment whereby stomata were smaller and denser (Figure 7). The stomatal arrangements (larger and less dense) in HHLS treatment are comparable to those in plants grown under high soil moisture conditions. However, unlike the

high soil moisture plants, the leaves in the HHLS were significantly shorter (Figure 13), so the larger and less dense stomatal arrangements cannot be due to epidermal cell expansion of larger leaves (Nejad and Van Meeteren, 2005). The stomatal arrangements could therefore be a direct response to the high humidity conditions. The response could be turgor driven, as mentioned previously in maize, though unlike maize, this turgor response could be humidity driven rather than dominated by soil water potential, perhaps through reduced stomatal conductance.

In low soil moisture and low humidity conditions (LHLS) wheat stomata are smaller and denser which could be attributed to the smaller leaves in this treatment and reduced epidermal cell expansion (Nejad and Van Meeteren, 2005) and also a reduction in leaf turgor causing reductions in cell size and increased density (Rodriguez-Dominguez *et al.*, 2016; Kim *et al.*, 2018), as discussed previously in maize.

The turgor driven changes in stomatal morphology could explain the larger and less dense wheat stomata in high humidity low soil moisture (HHLS) conditions since leaf size cannot account for these changes. Leaf turgor can be maintained through decreases in transpirational water loss (Mizrahi, Blumenfeld and Richmond, 1970). As high humidity leads to low VPD, and can therefore reduce transpirational demand (Shamshiri *et al.*, 2018), wheat may perceive this reduced VPD (Wheeler and Stroock, 2008) directly and it is enough to maintain leaf turgor despite low soil moisture conditions, and therefore maintain larger stomata with a less dense arrangement. Furthermore, the smaller and denser stomata in LHLS are

considered to favour plant growth in water-stressed environments due to their ability for rapid closure and subsequent greater control to mitigate significant water losses (Meinzer *et al.*, 2017). Moreover, plants can maximise stomatal conductance (g_{smax}) through a reduction in stomatal size and an increase in stomatal density (Bertolino, Caine and Gray, 2019). As such, the larger and less dense stomatal arrangement in low soil moisture conditions when humidity was high, would explain the reduced g_{smax} values in HHLS compared to LHLS (Figure 9). The LHLS treatment boasted the highest g_{smax} values, the highest stomatal densities, and the smallest stomata amongst all the treatment conditions. This was expected due to the water limiting stresses imposed on the plants from both high VPD and reduced soil water content.

Both low humidity and low soil moisture resulted in increased closure of wheat stomata. Interestingly, in low soil moisture conditions, high humidity did not result in the opening of stomata -as seen in maize-, suggesting that soil moisture is the dominant driver of stomatal opening/closing in wheat, not humidity. This could be due to the anisohydric nature of wheat whereby leaf water potential decreases with increased evaporative demand during the day and is dependent on soil water status (Schultz, 2003), and therefore wheat stomatal aperture shows greater sensitivity to soil water status. On the other hand, maize is isohydric, maintaining constant leaf water potential levels throughout the day and is not dependent on soil moisture status, therefore could lend itself to increased sensitivity to humidity (VPD) which would explain the opening of maize stomata in high humidity low soil moisture conditions as previously discussed.

2.5.2.2 Photosynthetic Efficiency

With regards to the photosynthetic parameters (Figure 11), unlike maize, there was more variation in wheat. This is not surprising considering the C3 nature of wheat, whereby stomatal conductance and photosynthesis tend to be more sensitive due to the need to present a higher stomatal aperture for CO_2 diffusion, increasing the potential for transpiration. This is reflected in observed changes in stomatal morphology and aperture, of which there were considerable treatment driven variations.

In wheat, there was a clear humidity driven response to lower ΦPSII and subsequently increase ΦNPQ, but only when soil moisture is high (Figure 11). It is possible that in the low soil moisture conditions when a majority of wheat stomata are closed, the plant is operating with great photosynthetic efficiency whereby any changes to VPD would have little effect on the photosynthetic capacity of the plant due to the soil water limiting conditions. Wheat is sensitive to drought and therefore when growing in soil water limiting conditions, seeks to minimise water loss through stomatal closure which inevitably lowers the photosynthetic capacity of the plant. Whereas in high soil moisture conditions, whereby soil water is not limiting, high VPD caused by low humidity conditions could be increasing stomatal conductance, with just over 50% of stomata remaining open, thus leading to increased photosynthetic operating capacity in the LHHS treatment. The reduction in ΦPSII when humidity is high (HHHS), for wheat, could be a result of the reduced VPD, lowering stomatal conductance resulting in reduced photosynthetic efficiency.

The predominantly closed stomata in the low soil moisture conditions not only could have influenced photosynthetic parameters but also that of leaf temperature (Figure 11). The LHLS treatment had the warmest wheat leaves whereby a majority of the stomata were closed and not contributing to evaporative cooling (Ball, Cowan and Farquhar, 1988). Such an increase in temperature could have further implications for net plant productivity. However, the warmer leaves in LHLS did not appear to impact photosynthetic efficiency as ΦPSII values were similar to the HHLS and HHHS treatments (both with cooler leaves compared to LHLS) (Figure 11). A possible reason for the lack of effect on ΦPSII was that the leaves did not get warm enough to significantly impact Rubisco activity. Rubisco inactivation in wheat is said to occur at 30°C (Feller, Crafts-Brandner and Salvucci, 1998), such temperatures were not reached by the wheat leaves in the LHLS treatment.

Through the analysis of the photosynthetic properties of wheat, the LHHS treatment had the highest ΦPSII which suggests that this treatment could be experiencing high rates of transpiration due to the low humidity (high VPD) conditions. These processes could be supported by the ample soil water reserves which could, in turn, could support higher rates of stomatal conductance. This all would imply that the LHHS treatment is resulting in the most productive wheat plants, a notion that is supported by the highest overall fresh and dry weights found in LHHS treatment (Figure 13).

2.5.2.3 Biomass

In stark contrast to maize, whereby root biomass remained relatively constant, the wheat root system is exhibiting great plasticity to the prevailing environmental

conditions (Hepworth, *et al*., 2016) and showed considerable sensitivity to soil moisture, and humidity when soil moisture was high (Figure 13). As low soil moisture resulted in significantly reduced dry weight, echoing findings from other wheat root studies (Morita *et al*., 1997), high soil moisture led to increased root biomass production, which was further increased through low humidity (high VPD) conditions (Figure 13). This larger investment in root biomass could be to create a root system capable of supporting larger productive wheat plants (Agathokleous *et al.*, 2018) through deeper penetration, increased root surface area, and wider exploration helping to unlock previously inaccessible soil water and nutrient reserves (Bauerle *et al.*, 2008; Nibau, Gibbs and Coates, 2008; Henry *et al.*, 2012). The productive plants in the LHHS treatment also had significantly longer primary and seminal roots (Figure 15), supporting the idea of more widespread foraging required to support the productive plant. Though a plant must have the means to support the extensive root growth in high soil moisture conditions as the metabolic costs of soil exploration can exceed half of daily photosynthesis, (Lambers, Atkin and Millenaar, 2002b). The wheat plants in high soil moisture conditions also exhibited the greatest shoot dry weights and longest leaves, which means they have a greater amount of photosynthetic area available to support the extensive root growth.

Wheat shoot biomass, on the other hand, appears to be responding solely to soil moisture conditions with reduced shoot biomass when soil moisture is low (Figure 13). This could be a result of both reduced stomatal conductance and photosynthesis in water deficit conditions (Shamshiri *et al*., 2018; Taiz *et al*., 2014), and also the direct allocation of the limited resources to root growth at the

cost of reduced shoot growth. Echoing findings from maize, the root:shoot dry weight ratio is highest in low soil moisture conditions, unlike maize, there is an additional humidity effect whereby during low soil moisture conditions high humidity reduced the root:shoot ratio (Figure 13) which is likely to have been caused by the slightly higher shoot dry weights in HHLS conditions compared to LHLS. This could be possible evidence of high humidity ameliorating some of the effects of low soil moisture conditions, though changes to shoot and root dry weight is minimal, the reduced ratio would imply high humidity is causing a reduction in the investment in root growth over shoot growth when soil moisture is low.

2.6 CONCLUSION

Table 3. Summary of main findings for both maize and wheat with regards to stomatal morphology, photosynthetic capacity and biomass production in response to humidity and soil moisture treatment effects.

	Maize	**Wheat**
Stomata	Stomata appear more humidity sensitive	Stomatal response to humidity only when soil moisture is low
Photosynthetic capacity	Treatments have little influence on photosynthetic parameters	Humidity effect on photosynthetic efficiency only when soil moisture is high

Biomass	Biomass production affected by soil moisture, not humidity	Very soil moisture sensitive, with roots affected by humidity only when soil moisture is high
	Low soil moisture causes increased investment in root growth over shoot growth.	

Hypotheses revisited:

1. Stomatal size will increase, and stomatal density will decrease in high humidity conditions.

We accept this hypothesis for maize but only during high soil moisture conditions, and for wheat but only under low soil moisture conditions.

2. More stomata will be open in high humidity conditions compared to low humidity, regardless of soil moisture.

We accept this hypothesis for maize but reject it for wheat.

3. High humidity will increase ΦPSII values (higher photosynthetic capacity)

We accept this hypothesis for maize but reject it for wheat.

4. Shoot biomass (dry weight) will increase in high humidity

We reject this hypothesis for both maize and wheat.

5. Root biomass (dry weight) will be unaffected by humidity.

We accept this hypothesis for maize but reject for wheat under high soil moisture conditions.

This chapter has highlighted the importance of investigating the effects of both humidity and soil moisture, as some humidity effects are dependent on the soil moisture conditions and vice versa, particularly with regards to wheat. Furthermore, this chapter has highlighted the potential for high humidity to ameliorate low soil moisture stresses and that different species and photosynthetic strategies (C3/C4) can influence the degree of sensitivity to changes in humidity and soil moisture conditions. Overall, the effects of humidity on maize appeared more soil moisture independent whereas the effects of humidity on wheat appeared more soil moisture dependent. However, this experiment was only carried out on young plant material, to be able to determine how the respective species will respond as a whole to these treatment conditions, longer-term experiments on different stages on plant development will need to be carried out.

3 THE EFFECTS OF ATMOSPHERIC HUMIDITY AND SOIL MOISTURE ABSISIC ACID CONCENTRATIONS IN LEAF AND ROOT TISSUE

3.1 INTRODUCTION

Since the dawn of land flora (~470Ma), plants have been forced to adapt to an array of terrestrial environmental conditions and stresses (e.g. drought, salinity, freezing,) during their growth and development on land (Ligrone, Duckett and Renzaglia, 2012). The detection and subsequent response to such stresses are crucial to their long-term survival and reproduction efforts (Zhang *et al.*, 2006). As such, a plethora of stress detection and response mechanisms have been developed across the plant kingdom with phytohormones playing a pivotal role.

Phytohormones are a group of small, naturally occurring molecules which influence plant processes at very low concentrations (Davies, 1995, 2004), they are the 'chemical messengers' relaying information throughout the plant and managing cellular responses that aid plant growth and development. To date, nine categories of phytohormones have been identified: auxins, cytokinins, gibberellins, abscisic acid, ethylene, brassinosteroids, salicylates, jasmonates, and strigolactones (Su *et al.*, 2017). One key stress response hormone we will be focussing on during this chapter is that of abscisic acid (ABA).

The biosynthesis of ABA is sensitive to changes in environmental conditions, leading to rapid accumulation (Zhang *et al.*, 2006) in roots, xylem and shoots (Davies and Zhang, 1991). The hormone is linked to numerous plant development

processes including seed dormancy, organ growth and development, and stomatal closure (Yoshida *et al.*, 2019). ABA is, therefore, a crucial hormone to study when investigating plant responses to environmental stresses, in particular water deficit and drought tolerance (Seo and Koshiba, 2002) and high VPD (low humidity) conditions. During water deficit conditions (albeit dry soil or high VPD driving high transpirational demand and subsequent water loss), a plant must respond accordingly to maintain tissue water potential, whilst minimising the negative impact on photosynthesis and subsequent productivity. A trade-off between carbon gain and water loss drives a variety of plant responses and behaviours designed to ensure the plant not only remains alive and functional during adverse conditions but also maintains high productivity in terms of carbon gain.

Amid dry soil conditions, roots are on the frontline belowground, detecting low soil moisture conditions and conveying a signal to the shoots to induce stomatal closure. The true nature of the signal has been debated over the years, with some claiming it is hydraulic based, others that ABA is transported around the plant. The most recent consensus is that it is a combination of the two, both playing an important role in plant response to drought conditions. For a comprehensive review on signalling see Buckley (2019). Whilst, amid dry air conditions where low humidity drives high VPD, leaves are on the frontline aboveground. Independently capable of producing their own ABA and potentially transporting foliar derived ABA down to the roots. This adds to the complexity of ABA synthesis, transport, and subsequent responses of the plant to above and belowground environmental stresses (Davies, Wilkinson and Loveys, 2002).

3.1.1 ABA movement

The literature suggests that during soil drying conditions, ABA biosynthesis increases within the plant, leading to a subsequent rise in ABA detected in the roots, xylem sap and leaves (Davies and Zhang, 1991). ABA concentrations in the xylem suggest that the ABA signal is produced in the roots then transported via the xylem to the transpiring leaves, a relatively one-way process (Zhang *et al.*, 2006). However, even back in 1893, Francis Darwin demonstrated stomata can close in direct response to a drop in atmospheric relative humidity, regardless of signals from the roots (Bauer *et al.,* 2013). The direct response of the leaves is thought to be a result of guard cell-autonomous ABA production, and therefore should be considered as another ABA biosynthesis pathway that can respond to changes in environmental conditions (Bauer *et al*., 2013). There is also evidence that ABA is produced in the leaves and transported down to the roots via the phloem (Neales and McLeod, 1991; Liang, Zhang and Wong, 1997; Wilkinson and Davies, 2002). Such a process can increase phloem-sourced ABA in the xylem sap by 25-30% (Neales and McLeod, 1991). There appears to be a great deal of free ABA biosynthesis, movement, and recycling around the plant system, from roots to shoots, and vice versa. ABA, therefore, has the potential to influence both aboveground and belowground organs in response to environmental factors such as atmospheric humidity and soil moisture content.

3.1.2 The Effects ABA on Roots

Root cells continually synthesise low levels of ABA, even in optimum well-watered conditions (Wilkinson and Davies, 2002), maintaining a basal level of ABA. During these ‘normal’ conditions, ABA is considered crucial for the growth of plant organs such as the primary root (Spollen *et al.*, 2000) and also post-

germination seedling development (Cheng *et al.*, 2002). Moreover, basal levels of ABA production are required to facilitate effective hydrotropic responses of root growth, to ensure the efficient exploration of the soil environment for water (Yoshida *et al.*, 2019), as roots of ABA-deficient and ABA-insensitive mutants have shown reduced hydrotropic response (Harris, 2015).

When a plant experiences environmental stresses such as soil drying, the strength of the ABA signal and subsequent responses can depend on a variety of factors including the rate of ABA biosynthesis, external influencing factors such as rhizospheric sourced ABA (Wilkinson and Davies, 2002), degradation, storage capacity, and xylem flow rates, which are ultimately driven by transpiration (Davies and Zhang, 1991). Despite increased root ABA production in dry soil conditions (Wilkinson and Davies, 2002), not all of the ABA enters the xylem, for transport to the rest of the plant. Firstly root cells are capable of storing or degrading ABA as well as taking it up as it passes by on route to the xylem (Wilkinson and Davies, 2002). However, ABA degradation during dry soil conditions slows down. A study on *Zea mays* (Liang, Zhang and Wong, 1997) extended the half-life of ^{3}H-ABA supplied to maize roots from 1.15 to 2.27 h through the drying of the surrounding soil, consequently ensuring more ABA is available to penetrate the xylem, and move to other parts of the plant, as an ABA signal.

The strength of the ABA signal is ultimately dictated by the transpirational flow of water through the soil-plant-air continuum. If this flow strengthens via increased rates of transpiration, there is a greater influx of water flow across the

root, carrying root-derived ABA across to the xylem and towards the rest of the plant (Freundl, Steudle and Hartung, 2000). Furthermore, high concentrations of ABA in roots can lead to increased root hydraulic conductivity (Wilkinson and Davies, 2002). Studies have shown that high concentrations of ABA in roots can increase the flow of water into and across the root by initiating the opening of inwardly directed water channels known as aquaporins (Netting, 2000; Tyerman, Niemietz and Bramley, 2002), thus increasing the flow of ABA into the xylem and increasing the strength of the signal.

Nevertheless, ABA is more than just a root-to-shoot signal. Before the hormone makes its way to the shoots to induce stomatal closure it has a significant role to play in the roots. At high concentrations, ABA can cause reduced root growth, (Sharp and LeNoble, 2002; Harris, 2015) and a study on representative angiosperms: *Vicia faba* cv. Crimson Flowering (Fabaceae), *Zea mays* cv. Golden Bantum (Poaceae) and *Helianthus annuus* cv. Yellow Empress (Asteraceae), found foliar-derived ABA to promote root growth relative to shoot growth during water limiting conditions (McAdam, Brodribb and Ross, 2016). Nonetheless, such effects are species-specific as high root [ABA] can stimulate root elongation in water-stressed maize (Sharp *et al.*, 1994), and promote primary root growth (Saab *et al.*, 1990).

In addition, ABA losses to the rhizosphere also need to be considered when assessing ABA concentrations and movement throughout the plant system. ABA can also be lost to the rhizosphere via diffusion when root ABA concentrations are high. A study on the rhizosphere surrounding maize observed increased ABA

concentrations very closed to the root (<2mm) during severe drought conditions (Müller, Deigele and Ziegler, 1989). Such results could be interpreted that during extreme drought conditions there was substantial participation of the roots in supplying hormones such as ABA to the rhizosphere. ABA synthesis in the root is crucial for keeping the ABA concentration stable in plants and ensuring stomata are equipped to respond to changes in environmental conditions. consequently, roots need to continually produce ABA so to keep in control and not lose all ABA to the xylem or rhizosphere.

3.1.3 The Effects of ABA on Leaves

Considering ABA can be transported to, biosynthesised in, and transported from the leaves, it can have a significant impact on foliar physiology. High concentrations of the hormone lead to reduced cell elongation and leaf expansion but more notably, induced stomatal closure (Loveys and During, 1984; Davies and Zhang, 1991; Dodd, 2005) to mitigate water loss during stressful conditions, by reducing stomatal conductance and maintaining plant water status (Davies and Zhang, 1991). Closing stomata to curtail water loss can disproportionately affect carbon gain which could be costly to the plant therefore a fine balance between water loss and carbon gain is established, whereby plants maintain meticulous control.

3.1.4 Symplastic ABA Reservoir

Stomata do not need a large concentration of [ABA] to reach the guard cells to induce closure (Wilkinson and Davies, 2002), as such if they responded directly to xylem [ABA] they would remain permanently closed. Plants have therefore adopted a strategy to ‘buffer’ ABA signals, by storing ABA in a symplastic

reservoir (Wilkinson and Davies, 2002). This reservoir can sequester and/or catabolise ABA when it is full ABA moves into the apoplast where it induces stomatal closure (Wilkinson and Davies, 2002). Leaves, therefore, have a certain threshold at which they can store ABA concentrations when the threshold is reached, stomatal closure is induced (Wilkinson and Davies, 2002). This threshold or level of sensitivity is affected by numerous factors, including the strength (concentration) of the ABA signal, pH of xylem sap, and hydraulic based signals.

3.1.5 pH Sensitivity

It is widely accepted that pH increases in response to edaphic stresses such as drought (Wilkinson *et al.*, 1998), and should also be noted that increases to xylem sap pH have been observed in drying soils even when the shoot water status of the plant is maintained under a root pressure vessel (Schurr, Gollan and Schulze, 1992). Stomatal responses to xylem pH are variable, with some leaves exhibiting partial closure when alkaline buffers (pH >7) are applied (Wilkinson and Davies, 1997; Wilkinson *et al.*, 1998). Whilst others show no effect or the opposite, a study on an ABA deficient tomato mutant (*flacca*) (Wilkinson and Davies, 1997) showed that when leaves were detached and fed pH 7 buffers, there was no observed stomatal closure, and in some cases, transpiration increased. The lack of response in ABA-deficient mutants suggests that ABA response and pH go hand in hand, in terms of stomatal closure. An increase in xylem sap pH has been shown to reduce ABA sequestration in the symplastic reservoir (Wilkinson and Davies, 1997). As such more ABA reaches the apoplastic sites (Gollan, Schurr and Schulze, 1992) at the guard cells thus inducing stomatal closure (Wilkinson and Davies, 1997). The rise in pH can therefore be considered to raise the plant's 'sensitivity' to [ABA].

The effects of pH are not just confined to xylem sap. Direct increases in leaf sap pH have been linked to high VPD conditions (Davies, Wilkinson and Loveys, 2002; Wilkinson and Davies, 2002), which could then heighten stomatal response to ABA. An experiment on *Forsythia* × *intermedia* (cv Lynwood) found high VPD increased pH and caused stomatal closure, which correlated with increased bulk leaf (but not xylem) [ABA] (Davies, Wilkinson and Loveys, 2002), indicating the [ABA] was synthesised directly at the foliar sites. As such, sensitivity to ABA concentrations can be altered at the leaf level through changes in leaf pH, in response to VPD.

3.1.6 Hydraulic Sensitivity

Stomatal sensitivity to ABA is also considered to be driven by hydraulic signals whereby reduced water potentials results in heightened stomatal sensitivity to ABA signals (Tardieu and Davies, 1992), suggesting that epidermal water relations may act as a mediator of stomatal closure to ABA signalling (Tardieu and Davies, 1992). Also, high [ABA] at foliar sites not only induce stomatal closure but are also associated with the inhibition of shoot growth at low water potentials (Saab *et al.*, 1990). Furthermore, leaf turgor has been observed to decrease in response to high [ABA] in the shoots of nutrient-deficient wheat plants (Vysotskaya, Korobova and Kudoyarova, 2008).

ABA is, therefore, a vital plant hormone, able to influence a variety of physiological process in response to environmental stresses such as drought conditions. ABA signalling from roots to shoots and vice versa is dependent on several factors such as rates of biosynthesis, xylem sap pH, transpirational flow

and root hydraulic conductivity. Understanding how ABA is created and perceived within the plant system gives us an insight into how a plant can cope with water stress and where the stress is being 'felt' the most as higher concentrations of ABA in either roots or shoots can be indicative of where the site of most stress is (Hu *et al.*, 2016). This chapter aims to investigate whether belowground water stress (drought) has as equally large impact on above-ground processes (e.g. stomatal aperture) as aboveground water stress (low humidity, high vapour pressure deficit (VPD). As such, investigating how ABA responses differ under various humidity and soil moisture treatment conditions could help to decouple the effects of soil moisture and humidity on plant physiology, whilst providing an insight into whether high humidity conditions have the potential to reduce plant stress caused by dry soil conditions.

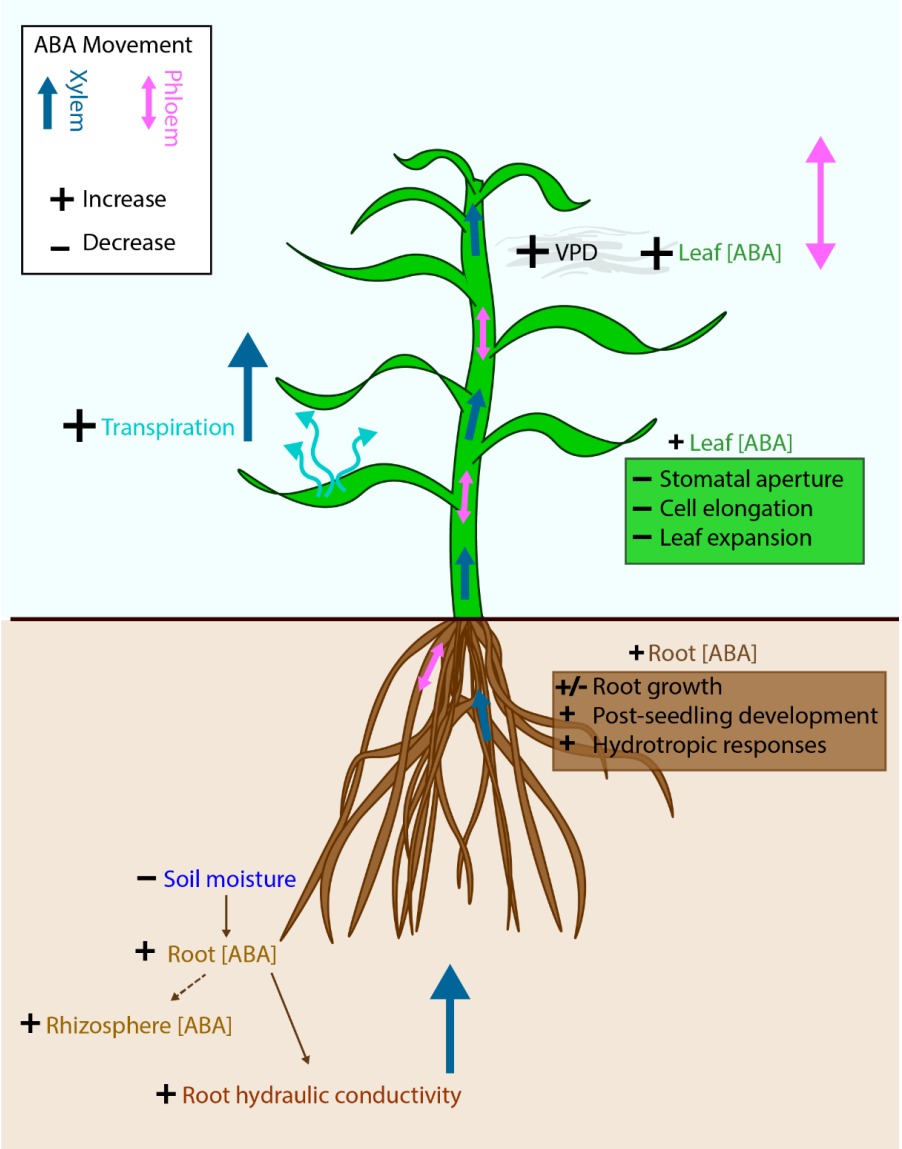


Figure 1. Schematic representing the main processes affecting ABA movement within a plant and the effects of increased leaf and root ABA concentrations on the respective organs. The response of ABA movement in the xylem and phloem vessels are represented with coloured arrows, larger arrows represent increased movement with + and – representing increases or decreases to respective processes. The dashed arrow between root [ABA] and rhizosphere [ABA] represents a potential process that could occur providing root [ABA] is high enough.

3.2 AIMS AND OBJECTIVES

Most studies do not take humidity into account when investigating the effects of drought (dry soil) on plant physiology and ABA hormone regulation.

This study will focus on ABA responses to environmental stresses relating to water availability both aboveground (relative humidity/VPD) and belowground (soil moisture content).

3.2.1 Hypotheses to be Tested

1. Low humidity (high VPD) will lead to higher foliar ABA concentrations, regardless of soil moisture content.
2. Low soil moisture will lead to increased root [ABA] regardless of humidity treatment.
3. The root:leaf [ABA] ratio will be lower in low humidity conditions.

3.3 METHODS

3.3.1 Plant Material and Experimental Design

24 maize (*Zea mays*) and 24 wheat (*Triticum aestivum* cv. Paragon) seeds were germinated in 2L pots, packed at a 1.3g cm^{-3} bulk density with sandy loam soil collected from a field site in Bunny, Leicestershire, UK (Longitude=-1.12608866, Latitude=52.86098725). During germination, soil moisture content for all treatments was maintained at 70% field. Once germinated, the pots were randomly arranged in a custom chamber detailed in Chapter 2, that was situated in a temperature (heating and vents) glasshouse. Six reps of maize and wheat were subjected to the following treatment conditions (Table Table 1). Due to plant death, the biological rep of samples for maize is n=5 for HHHS and LHHS, and n=6 for HHLS and LHLS. For wheat n=6.

Soil moisture was maintained with regular watering to weight, to achieve treatment conditions. Air temperature and relative humidity were recorded every hour, in both the high and low humidity section of the chamber, using a Fisher Scientific Traceable Humidity/Temperature. Dew-Point Meter (Fisher, UK), recorded values are presented in Figure 2 and Figure 3. Vapour pressure deficit (VPD) values were calculated using recorded air temperature and humidity data following two equations from. (Jones, 1992), that are presented in the Chapter 2 methods section (2.3).

Table 1. Treatment growth conditions for the three weeks of growth of maize and wheat. Relative humidity and temperature measurements were recorded using a Fisher Scientific Traceable Humidity/Temperature. Dew-Point Meter (Fisher, UK). Day refers to 06:00 – 18:00 and night (18:01 – 05:59). Soil moisture treatment was maintained with regular watering to weight.

Treatment	**Relative Humidity (%) (day/night)**	**Soil moisture (field capacity %)**	**Average Temperature °C (day/night)**	**Calculated Air Vapour Pressure Deficit (VPD_{air} kPa) (day/night)**
High Humidity High Soil Moisture **(HHHS)**	85.11/96.59	70	29.75/19.31	0.8/0.08
High Humidity Low Soil Moisture **(HHLS)**	85.11/96.59	30	29.75/19.31	0.8/0.08
Low Humidity High Soil Moisture **(LHHS)**	31.59/32.1	70	28.04/20.2	3.25/1.64
Low Humidity Low Soil Moisture **(LHLS)**	31.59/32.1	30	28.04/20.2	3.25/1.64

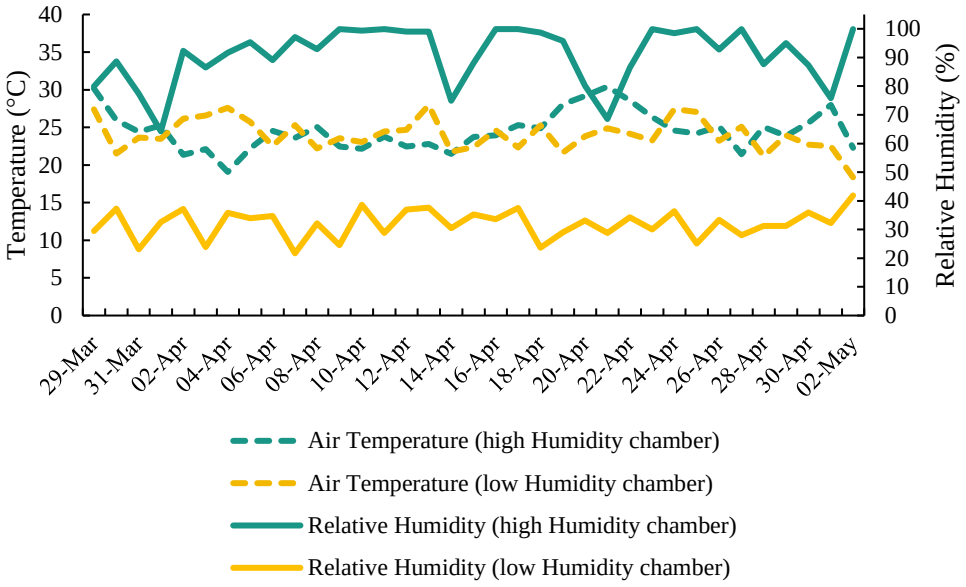


Figure 2. Daily averages of the growth conditions in the high humidity and low humidity chambers, throughout the experiment. Measurements recorded using a Fisher Scientific Traceable Humidity/Temperature. Dew-Point Meter (Fisher, UK). Green (■) lines represent recordings in the high humidity chamber, whilst yellow (■) lines represent recordings in the low humidity chamber.

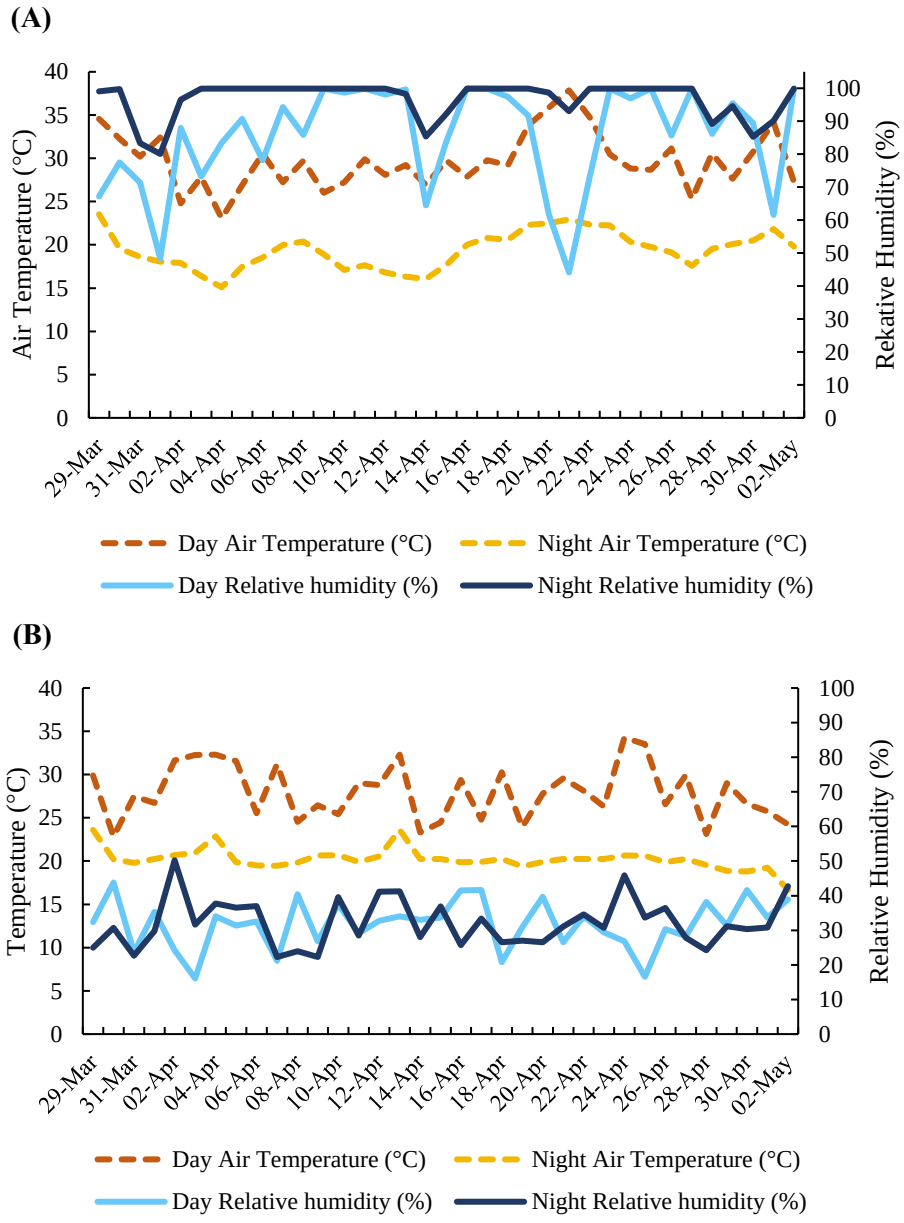


Figure 3. Day and night average temperature and relative humidity in the high humidity growth chamber (A) and the low humidity chamber (B). Measurements were recorded using a Fisher Scientific Traceable Humidity/Temperature. Dew-Point Meter (Fisher, UK). Day refers to 06:00 – 18:00 and night 18:01 – 05:59.

3.3.1.1 Collection of samples for ABA analysis

Before samples were harvested, 1.5ml Eppendorf tubes were prepared. A small pinprick hole was placed in the lid so to enable gas to escape during freezing and freeze-drying processes, and the empty Eppendorf tube was submerged in liquid

nitrogen (~3 seconds), to help speed up the freezing of the samples. Leaf and root samples (10-20mg) were taken from maize and wheat plants three weeks after germination.

Leaf samples:

Three leaf samples were taken in the morning (9 am-10 am) from each plant, samples were collected from the longest unfurled, second-longest unfurled, and third-longest unfurled leaves. If leaves were excessively large, a ~10 cm cutting was taken from the middle portion of the leaf (removing the tip and base of the leaf). This was so the sample could fit into the 1.5ml Eppendorf tubes that had been pre-frozen in liquid nitrogen.

Root samples:

Three seminal root samples were taken from each plant. Due to the young plant material, the whole root was able to be collected and placed into the 1.5ml Eppendorf.

3.3.1.2 Freezing

Once excised from the plant, samples were placed into a stainless-steel vessel of liquid nitrogen. When the harvest of samples was complete, Eppendorf tubes containing the plant material were transferred and stored in the -80°C freezer, whilst awaiting freeze-drying.

3.3.1.3 Freeze-drying

Samples were placed in a freeze drier (Labogene Scanvac coolsafe 55-9) for 96 hours. After 96 hours samples were checked and if any condensation was found on the inside of the Eppendorf tube, samples were placed in the freeze-drier for a second time and left for a further 96 hours or until no further condensation was

present. Once completely freeze-dried samples were placed into new 1.5ml Eppendorf's that were airtight (no pinprick hole present).

3.3.1.4 Grinding

Finely ground plant material powder was required for hormone analysis. Firstly, samples were cut into smaller pieces and placed back into 1.5ml Eppendorf tubes then a ball mill (Qiagen, TissueLyserII) was used to grind the samples (30Hz for 4 minutes, check powdered state then repeat if necessary). Once the samples resembled a fine powder 10-20mg of the sample was weighed out and placed in 1.5ml Eppendorf tubes ready for ABA analyses.

3.3.1.5 ABA Analysis

Samples were sent to Lancaster University where they were analysed by Hend Mandour, following ABA extraction methods described in Quarrie *et al.*, (1988).

3.4 RESULTS

All statistical analyses were carried out in Genstat 20th Edition. Treatments and effects were compared using a general ANOVA with main effects of soil moisture and humidity tested as well as any significant interaction between the two at the 5% level. When significance was detected, post hoc Fisher's least significant difference test was carried out.

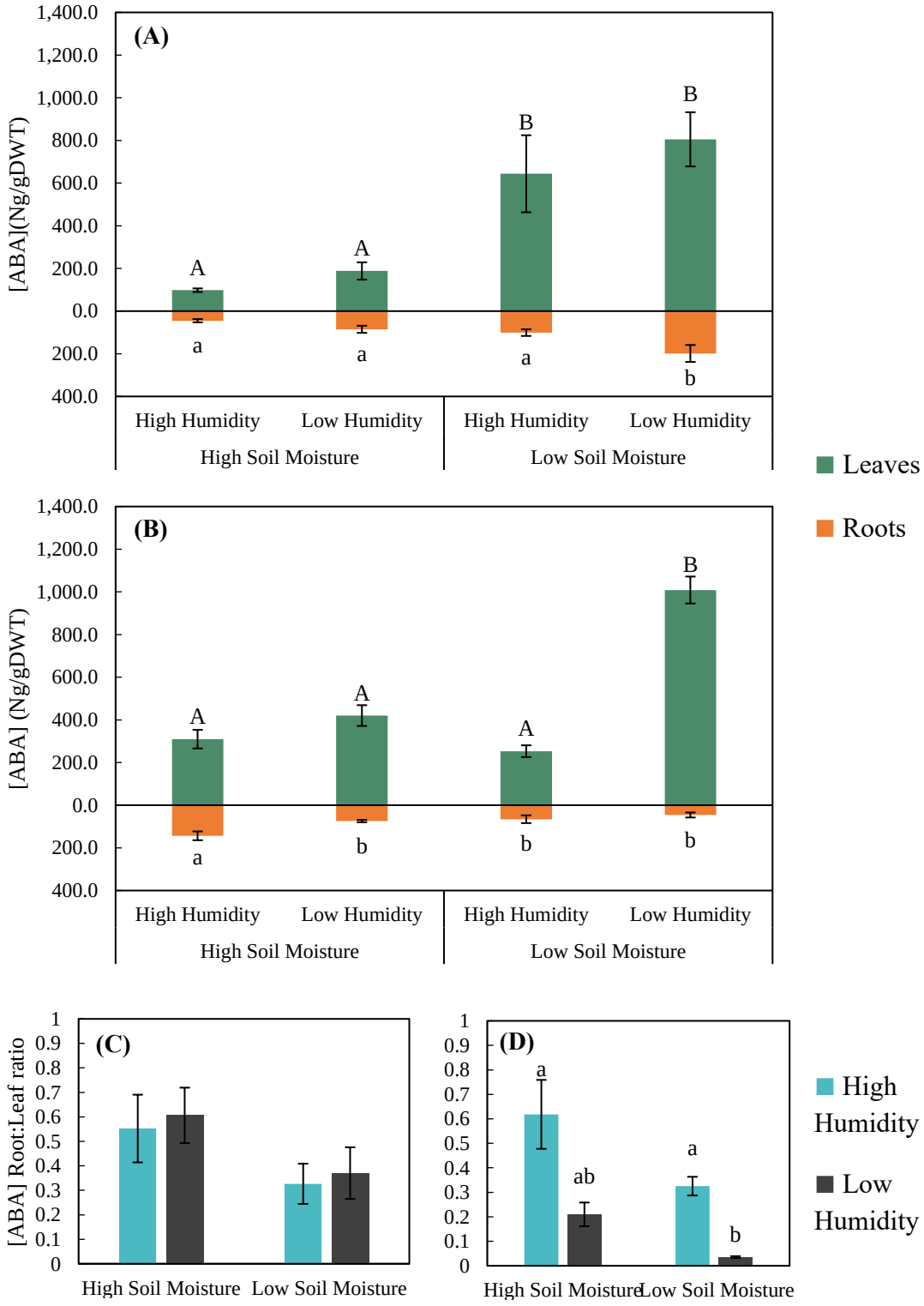


Figure 4. The concentration of ABA (Ng/g dry weight) in the leaves and roots of maize (A) and wheat (B), and the [ABA] root:shoot ratio for maize (C) and wheat (D), three weeks after germination then growth in the following treatment conditions High Humidity High Soil Moisture (maize n=5, wheat n=6), Low Humidity High Soil Moisture

(maize n=5, wheat n=6), High Humidity Low Soil Moisture (maize n=6, wheat n=6) and Low Humidity Low Soil Moisture (maize n=6, wheat n=6). Means are presented with error bars representing ± SE. Different letters present significance at the 5% level after the post-hoc Fisher's unprotected least significant difference test. In panels A and B, different uppercase letters compare leaf ABA concentrations between treatments and different lowercase letters compare root ABA concentrations between treatments.

The concentration of ABA in the leaves of maize (Figure 4 A) was significantly higher in low soil moisture conditions ($P<0.001$), regardless of humidity treatment. Whereas maize roots were significantly affected by both soil moisture content ($P<0.001$) and humidity ($P=0.008$), during low soil moisture conditions, ABA concentrations were significantly higher under low humidity conditions.

With regards to wheat (Figure 4 B), foliar ABA concentration was significantly affected by both humidity ($P<0.001$) and soil moisture ($P<0.001$), as well as a significant interaction between soil moisture and humidity ($P<0.001$).During low soil moisture conditions, high humidity resulted in [ABA] comparable to concentrations in wheat grown in high soil moisture conditions. Wheat root ABA concentration was significantly affected by both soil moisture ($P=0.007$) and humidity ($P<0.001$), during high soil moisture conditions, ABA concentration was significantly higher during high humidity conditions.

The analyses of the ABA concentration root:leaf ratio (Figure 4 C and D), indicated the proportion of ABA found in the roots compared to the leaves. Maize ABA root:leaf ratio (Figure 4 C) was not significantly affected by either soil moisture ($P=0.065$) or humidity ($P=0.28$), there was a relatively constant root:leaf

ABA ratio across all treatments. Wheat ABA root:leaf ratio (Figure 4 D), on the other hand, was significantly lower in low humidity conditions ($P<0.001$). Wheat had a significantly higher proportion of [ABA] in the leaves compared to the roots under low humidity conditions. In both maize and wheat, there was greater [ABA] in the leaves than the roots, across all treatments.

3.5 DISCUSSION

Maize and wheat respond differently in terms of the distribution of [ABA] between shoots and roots when exposed to different humidity and soil moisture regimes. This chapter highlights the need to investigate both humidity and soil moisture as potential influencers of [ABA] as plant responses (both in the shoot and root) vary depending on the treatment conditions.

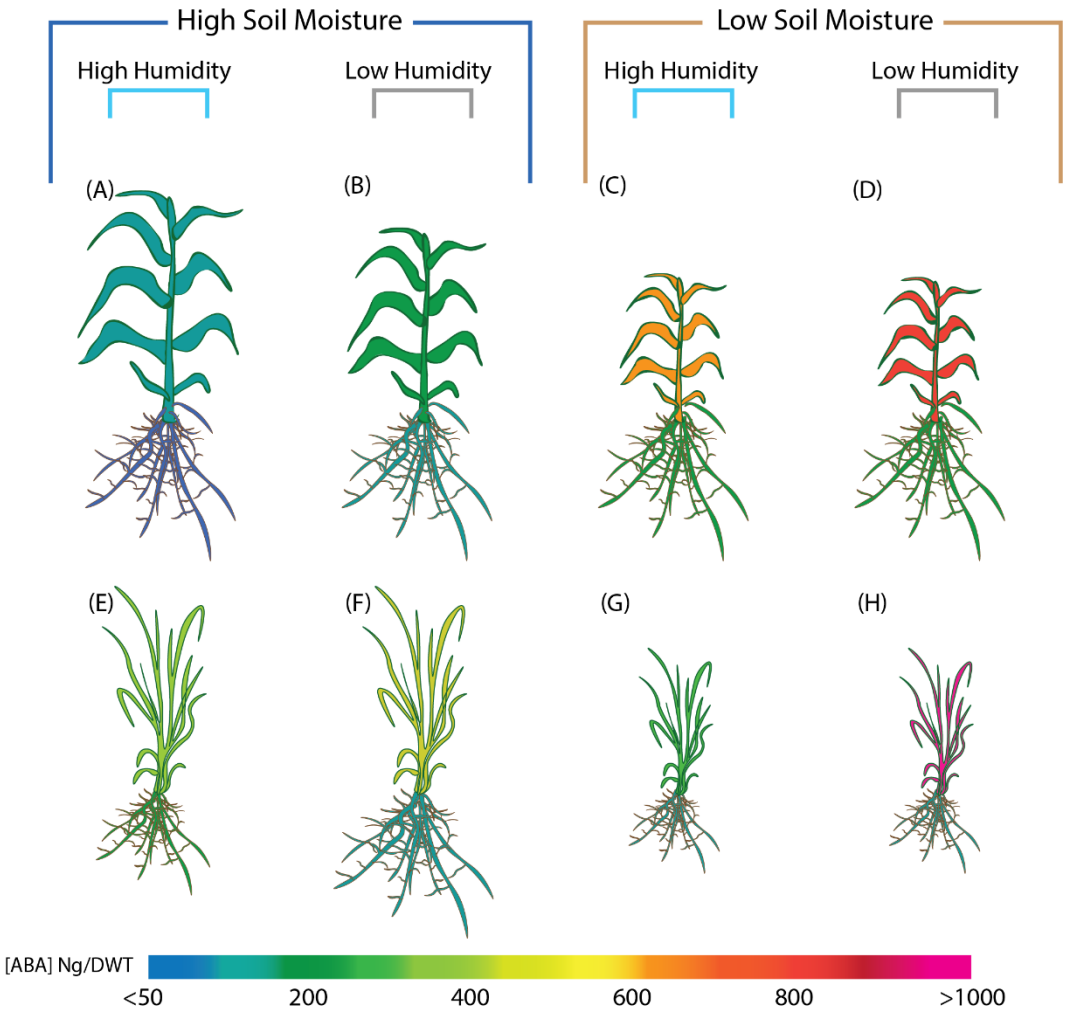


Figure 5. A schematic representing the concentration of ABA (Ng/DWT) in maize (A, B, C, D) and wheat (E, F, G, H) shoot and root biomass, in plants grown in the four treatment conditions. High Humidity High Soil moisture (A) maize and (E) wheat, Low Humidity High Soil moisture (B) maize and (F) wheat, High Humidity Low Soil moisture (C) maize and (G) wheat, and Low Humidity Low Soil moisture (D) maize and (H) wheat. Not to scale, but the relative sizes of roots and shoots are presented as per biomass dry weight results from Chapter 2.

Hypotheses revisited:

1. Low humidity (high VPD) will lead to higher foliar ABA concentrations, regardless of soil moisture content.

We reject this hypothesis for both maize and wheat. Maize shoot [ABA] is not affected by humidity but is significantly higher in low soil moisture conditions. Whilst wheat shoot [ABA] is only significantly higher in low humidity when soil moisture is also low.

2. Low soil moisture will lead to increased root [ABA] regardless of humidity treatment.

We reject this hypothesis for both maize and wheat. Both species' root [ABA] response to soil moisture is also dependent on the humidity. Whilst maize root [ABA] is significantly higher in low humidity and low soil moisture conditions, interestingly wheat is the opposite, with significantly higher root [ABA] in high humidity high soil moisture conditions.

3. The root:leaf [ABA] ratio will be lower in low humidity conditions.

We reject this hypothesis for maize as there were no significant treatment effects on the root:leaf [ABA] ratio. However, we accept the hypothesis for wheat.

The high concentrations of ABA in leaves of maize plants grown in low soil moisture conditions are comparable with the literature (Sanguineti *et al.*, 1999; Bahrun *et al.*, 2002; Giuliani *et al.*, 2005), with upregulation of ABA synthesis in leaves is commonly reported (Vysotskaya, Korobova and Kudoyarova, 2008),

driven by responses to environmental stimuli such as high VPD (Bauer *et al*., 2013) and dry soil conditions (Sanguineti *et al.*, 1999; Bahrun *et al.*, 2002; Giuliani *et al.*, 2005; Saradadevi *et al.*, 2014). However, the significantly higher leaf [ABA] in low soil moisture conditions could not only be due to the upregulation of ABA production at foliar sites but also from root-derived ABA, transported up through the xylem. Previous studies have observed increased levels of ABA recorded in the xylem during soil drying events (Wilkinson and Davies, 2002; Davies, Kudoyarova and Hartung, 2005). As such, increased ABA concentrations in roots are associated with an increased hydraulic conductivity through the effects of ABA on aquaporin functioning (Netting, 2000; Tyerman, Niemietz and Bramley, 2002), thus leading to potentially increased water transport through the roots, carrying more ABA (a stronger signal) to the shoots, whereby higher [ABA] is recorded. The reported [ABA] was conducive to findings in previous literature, whereby well-watered leaf [ABA] in maize lies around 100-200ng g^{-1} DW, and water-stressed (droughted) leaf [ABA] from around 500 to over 1000ng g^{-1} DW (Sanguineti *et al.*, 1999; Bahrun *et al.*, 2002; Giuliani *et al.*, 2005).

Despite maize shoots in low soil moisture conditions containing the highest [ABA], regardless of humidity, in Chapter 2 and Chapter 5 maize stomata remain open in HHLS and only show a larger proportion of closure in LHLS, whereas due to the high [ABA] in the shoots, we would expect both HHLS and LHLS to show prominent closure. This is possible evidence for the utilisation of the symplastic reservoir, whereby low humidity (high VPD) could be reducing the ability of the sequestration of ABA in the symplastic reservoir (Davies *et al*.,

2002) that could be higher in HHLS, thereby resulting in more ABA heading directly to the apoplastic microsites at guard cells thus resulting in the closure (in LHLS).

It is interesting that in maize low soil moisture, foliar sites have high [ABA] regardless of humidity whereas wheat sees a marked reduction in [ABA] when humidity is high. Perhaps the differing responses are associated with the various levels of isohydricity between maize (isohydric) and wheat (anisohydric).
As maize is isohydric, it has 'stricter' stomatal control and maintains a constant midday leaf water potential. So perhaps in low soil moisture conditions (regardless of humidity – VPD) maize accumulates ABA in the foliar sites, stored in the symplastic reservoir, ready to initiate stomatal closure to prevent water loss in dry soil conditions. However, despite high [ABA], in HHLS a majority of stomata remain open (see Chapter 5), with only a majority closed in LHLS (see Chapter 5). Perhaps it is the VPD that ultimately determines whether the plant should close stomata (release ABA from the symplastic reservoir into the apoplast so it can reach guard cells), a direct response to atmospheric conditions. The high humidity conditions (low VPD) could be reducing maize's sensitivity to the high foliar [ABA], therefore stomata remain open and only respond to high [ABA] when VPD is high (LHLS treatment). Therefore, potentially, maize stomata are more responsive to VPD compared to ABA.

Responses to VPD could also be driving changes to xylem sap pH which would have knock-on effects on the sensitivity of stomatal response to ABA. Xylem pH alkalisation can be triggered by environmental factors such as high VPD (Chaves

and Oliveira, 2004) and drought stress (Wilkinson *et al*., 1998). As such, the changing sequestration behaviour could be due to xylem sap becoming more alkaline (when both VPD is high and soil moisture low). An increase in xylem sap pH has been shown to reduce ABA sequestration in the symplastic reservoir (Wilkinson and Davies, 1997) thus diverting more ABA directly into the apoplast sites in guard cells and initiating stomatal closure in the LHLS treatment.

Wheat, as an anisohydric plant, has less stomatal control during periods of decreasing midday water potentials. This is partially consistent with the diminished difference in [ABA] between HS and LS treatments compared to maize Wheat could be more sensitive to [ABA] with regards to stomatal closure, but it is only produced under high VPD (low humidity conditions). Perhaps the low [ABA] in wheat foliar sites in HHLS conditions, is due to the reduced foliar synthesis of ABA when transpirational demand is low, to prevent a high proportion of unnecessary stomatal closure. We see more stomata open in HHLS compared to LHLS (see Chapter 5), which could suggest that stomata are sensitive and responding to ABA to a greater degree than leaf water potentials. Leaf [ABA] was conducive to findings in the literature for wheat (in HHHS, HHLS and LHLS) whereby [ABA] is around 100ng g^{-1} DW for well-watered conditions and up to 1000ng g^{-1} DW in drought conditions (Saradadevi *et al.*, 2014).

Though humidity had no significant influence on [ABA] in maize foliar sites, high humidity levels did lead to significantly lower [ABA] in roots. The reduced [ABA] in the maize roots under high humidity conditions when soil moisture was

low, could have been driven by the maintained transpiration rates under high humidity conditions. If the stomata were predominantly open during high humidity conditions, regardless of the soil moisture conditions, a relatively high level of transpiration could have been maintained, drawing up ABA from the roots, along the xylem (Freundl, Steudle and Hartung, 2000; Zhang *et al*., 2006).

Such stomatal apertures were recorded in Chapters 2 and 5, whereby high humidity caused a large proportion of maize stomata to remain open. Thus, leading to reduced root ABA, due to the rapid loading of ABA into the xylem and subsequent transport to foliar sites, during such conditions. This could also explain why under high soil moisture conditions, there was significantly lower [ABA] in maize roots compared to LHLS treatment, due to higher rates of transpiration (through a greater proportion of open stomata as seen in Chapters 2 and 5), leading to increased ABA flow from the roots to the leaves. However, the concentration of ABA in the plant system is dependent on both synthesis and breakdown (Seo and Koshiba, 2002), processes of which are influenced by environmental factors such as water stress and other growth regulators (Salazar, Hernández and Pino, 2015). It is possible that the lower [ABA] in maize roots grown under high soil moisture conditions and the HHLS treatment, is caused by an increased breakdown of the hormone, rather than lack of synthesis (or both). A limitation of the 'snapshot' measurements of [ABA] in this study, that that we only observe a single point of the net effects of these processes, it provides little information about the flux of ABA in the plant system.

On the other hand, wheat shoot [ABA] in the LHLS treatment reflects similarly high values to that of maize plants in the same treatment (LHLS). The high leaf [ABA] could be driven by increased biosynthesis at the foliar sites, increased transport from root-derived ABA and increased root uptake of soil ABA, all discussed above. Interestingly, unlike maize, during low soil moisture conditions, high humidity led to significantly reduced leaf [ABA] in wheat. The significantly lower leaf [ABA] found in HHLS treatment is comparable to the leaf [ABA] from plants grown under high soil moisture conditions both in this chapter and other wheat ABA drought studies in the literature (Saradadevi *et al.*, 2014).In this chapter, the leaves could be directly responding to the high humidity (low VPD), and high soil moisture treatment conditions thus reducing the synthesis of ABA at foliar sites, due to lack of 'stress' detected at the leaf level (lower transpirational demand and higher soil water availability, respectively), therefore any observable increases in [ABA] must be derived from the roots.

Though in the HHLS treatment, stomata may be responding to the soil water stress and closing despite low VPD, potentially reducing transpiration resulting in less water (and subsequent ABA) being drawn up through the xylem to foliar sites.

This idea of reduced transpiration is supported by data in Chapter 2 and Chapter 5 whereby a greater proportion of wheat stomata are closed in low soil moisture conditions, resulting in significantly reduced stomatal conductance (reported in Chapter 5). Though high [ABA] at foliar sites is associated with stomatal closure, and this is the case for the wheat LHLS plants, interestingly the HHLS treatment

also had a high proportion of closed stomata but relatively low [ABA] in the shoots. This could be evidence of a change in the symplastic reservoir capabilities when soil moisture is low, more ABA could be heading directly to the apoplastic sites where it induces stomatal closure, rather than being sequestered into the symplastic reservoir.

As previously discussed in maize, it is known that during edaphic stress such as drought, xylem pH increases (Wilkinson *et al*., 1998). As such, the changing sequestration behaviour could be due to xylem sap becoming more alkaline, as an increase in xylem sap pH has been shown to reduce ABA sequestration in the symplastic reservoir (Wilkinson and Davies, 1997). This can explain how we see a large proportion of closed stomata in both Chapter 2 and Chapter 5, for wheat in the HHLS treatment, even though shoot [ABA] is relatively low. A rise in xylem sap pH could be diverting what little ABA there is straight to the apoplast sites in the guard cells, effectively 'cutting out the middleman' (symplastic reservoir) and causing stomatal closure.

With regards to wheat roots, unlike maize, there is a significantly higher root [ABA] in plants grown under high soil moisture and high humidity (HHHS). The high root [ABA] under HHHS conditions, could be an indicator that the roots were experiencing near waterlogged conditions, due to the continually high soil moisture content. Waterlogging can induce ABA production in roots (Akhtar and Nazir, 2013), and an experiment on flooded pea plants (*Pisum sativum* L. cv. Feltham First) (Zhang and Davies, 1987) recorded significantly higher root [ABA] in waterlogged plants. However, the wheat in this chapter were not

standing in water and had no visual signs of root rot during end of experiment root washing procedures, this would indicate that the roots were not experiencing waterlogged conditions. Also, the lack of increase in [ABA] at foliar sites in HHHS treatment, could have been caused by potentially reduced rates of transpiration in low VPD conditions, reducing the net flow of ABA from the roots to the leaves, hence why no observable increase in leaf ABA was observed. However, in Chapter 5 we see significantly higher stomatal conductance in the HHHS treatment, which would imply that this is not the case.

Across all treatments, there was higher [ABA] (per unit dry weight) in leaves compared to roots, in both maize and wheat. To further explore the pattern of ABA distribution within the plant, the root:leaf [ABA] ratio was calculated. Interestingly, in wheat, low humidity led to significantly lower root:leaf ratios, whereby a far greater proportion of ABA (per unit dry weight) is found in the leaves compared to the roots, potentially brought about via the upregulation of foliar ABA biosynthesis during high VPD conditions (Wilkinson and Davies, 2002).

It is important to understand the distribution of ABA accumulation patterns, to predict long-distance ABA signalling to water stress (Hu *et al.*, 2016). A study on peanut *(Arachis hypogaea* L.) suggested that ABA biosynthesis is more pronounced at the site of most stress (Hu *et al.*, 2016). With regards to wheat, the far greater proportion of ABA found in the leaves of plants grown under low humidity (high VPD) would suggest that the foliar sites are experiencing the most stress through increased transpirational demand. The notion that dry air is a

greater stress to plants than dry soil is supported by a modelling study (Novick *et al.*, 2016) which showed VPD is a greater stress to plants from a variety of biomes (evergreen forest, deciduous broadleaf forest, croplands, grasslands and savannahs and shrublands) than dry soil conditions, and a study by Leuschner (2002) which concluded that VPD acts as a soil water independent growth controlling factor on temperate woodland herbs. It could be that the lower wheat [ABA] root:leaf ratios found in low humidity conditions, is evidence that wheat is experiencing a greater amount of stress in low humidity (high VPD) conditions, compared to low soil moisture conditions, supporting statements by previous studies (Leuschner, 2002; Novick *et al.*, 2016)

3.6 CONCLUSION

A range of processes is involved in modifying root and foliar derived ABA signals, as well as the stomatal sensitivity to such hormonal cues. This study has highlighted how sub-terranean and aerial influences can interact to impact plant development and functioning. Both maize and wheat respond differently, with maize shoots more heavily influenced by soil moisture and wheat shoots by humidity. The [ABA] root:leaf ratio suggests wheat leaves experience greater stress from low humidity than low soil moisture conditions. We are beginning to understand the whole plant signalling processes with regards to ABA, and with that, building on our understanding of why the intensity of such signals and subsequent responses vary between species and growth environments, such knowledge will go a long way in future breeding practices of tolerant varieties.

4 THE EFFECTS OF ATMOSPHERIC HUMIDITY AND SOIL MOISTURE ON ROOT ANATOMY AND LEAF CUTICLE CHEMISTRY

4.1 INTRODUCTION

Above and belowground plant physiology is intrinsically linked. Stomata, the gatekeepers, dictate stomatal conductance, which in turn affects transpirational demand and subsequent plant water potential. This resulting soil – plant - atmosphere water potential gradient is responsible for drawing up water and nutrients from the soil, to support plant growth and development, via the cohesion-tension theory (Jones, 1992). Though, realistically, such processes are far from straightforward and are regularly interrupted, whether it be from mechanical impedance from structures such as the Casparian strip, epicuticular waxes, and closed stomata, or disruptions to water flow via cavitation and embolisms in xylem, to name but a few. The exchange of molecules between the plant and the environment, is faced with myriad barriers and diffusional resistance, from the aerial to the subterranean organs. This chapter will focus on leaf cuticle chemistry and root anatomy, and how both could act as barriers and/or facilitators to water uptake, loss, and whole plant transport, under contrasting humidity and soil moisture conditions.

On the foliar frontline alongside stomata, at the plant-atmosphere interface, is the leaf cuticle. This protective lipophilic membrane facilitated plants invasion of the land in the early Palaeozoic, over 400Ma (Dominguez, Heredia-Guerrero and Heredia, 2011; Renault *et al.*, 2017; Salminen *et al.*, 2018). The main function of the cuticle is to protect against desiccation (Sánchez *et al.*, 2001), together with

the governance of gas exchange (Littlejohn *et al.*, 2015) through cuticular transpiration (Kerstiens, 1996; Riederer and Schreiber, 2001) Furthermore, the protective role of the cuticle has been acknowledged concerning defence against pathogens (Serrano *et al*., 2014) and insect attack (Eigenbrode and Jetter, 2002), and mitigating UV radiation exposure (Krauss, Markstädter and Riederer, 1997). Such a multi-functional nature is only permitted through the diverse structural and chemical landscape of the cuticle (Khayet and Fernández, 2012) which can vary considerably between species, growth conditions, and the physiological status of the plant (Fernández *et al.*, 2014, 2016; Guzmán-Delgado *et al.*, 2016). For a comprehensive review on the biophysical design of plant, cuticles see Dominguez, Heredia-Guerrero and Heredia (2011).

From a chemical perspective, the cuticle is made up of an array of compounds (Fernández *et al.*, 2016), including waxes, cutin and/or cutan, polysaccharides, phenolics, and mineral elements (Shepherd and Wynne Griffiths, 2006; España *et al.*, 2014; Guzmán-Delgado *et al.*, 2016). During soil drying, drought experiments, plant cuticle properties shift in favour of promoting hydrophobicity and reducing water loss through the cuticle. Increased wax content and cuticle thickness (Oosterhuis, Hampton and Wullschleger, 1991), higher proportions of aliphatic components (regarded as having chain lengths greater than C_{26}) (Macková *et al.*, 2013), are some ways in which the cuticle can respond to soil water deficit conditions. In addition to soil water status, the atmospheric water content in terms of relative humidity can also influence plant cuticle compositions (Itagaki *et al.*, 2014). High humidity (low VPD) conditions have been observed to drive changes to the cuticular wax composition, through reduced alkene content

and a less hydrophobic flavonoid profile, in silver birch leaves, subsequently reducing the hydrophobicity of the leaf surface (Lihavainen *et al.*, 2017).

There are a variety of methods employed to analysis plant cuticle properties and chemical compositions. Immuno-chemical studies have been successful at identifying the existence of cuticle constituents such as polysaccharides (Tenberge, 1992; Guzmán *et al.*, 2014) and cutin (Domínguez *et al.*, 2010; Kwiatkowska *et al.*, 2014), whilst chloroform is commonly used to extract epicuticular waxes for chemical composition analysis, such methods are described in Oosterhuis, Hampton and Wullschleger (1991). Nevertheless, a technique gaining traction in recent years is the use of Attenuated Total Reflectance Fourier Transform Infrared (ATR-FTIR) spectroscopy (Jardine *et al.*, 2019). The use of ATR-FTIR spectroscopy to investigate cuticle chemistry is a popular choice due to its non-destructive, efficient method which is appropriate for studies where only very small samples can be obtained (Heredia-Guerrero *et al.*, 2014) such as palaeobotany research (Olcott Marshall and Marshall, 2015; Jardine *et al.*, 2019). This technique was chosen for this chapter, due to the high throughput nature of the method, whilst producing a broad range of chemical information for each leaf sample in each of the humidity and soil moisture conditions. Through the application of ATR-FTIR techniques, we can detect a variety of organic constituents present on the leaf surface and shed light on the potential rates of diffusion across the membrane and the hydrophobicity of the leaf surface (Jardine *et al.*, 2019), as such, this compositional data may have useful ecological and botanical applications, from identifying key traits for breeding practices to aiding in the prediction of physiological plant responses to the changing climate.

Each leaf cuticle possesses a spectral chemical 'fingerprint' that can be observed using attenuated total reflectance Fourier Transform infrared (ATR-FTIR) (Ribeiro da Luz, 2006). ATR-FTIR is a technique which involves the application of infrared energy to a sample from a global light source, molecules in the sample absorb the energy, exciting them from ground state to vibrational state, which results in a characteristic spectrum (Baker *et al.*, 2014; Liu and Yu, 2016; Liu *et al.*, 2019). This technique displays complex absorption features that allow for the detection of chemical components and particular functional groups in isolated cuticles (Dominguez, Heredia-Guerrero and Heredia, 2011). It is also sometimes possible to deduce anatomical features such as wax thickness and the presence of trichomes (Ribeiro da Luz, 2006) from the leaf spectra. Studies applying this technique have shown that leaves from plants that have been exposed to higher levels of natural sunlight frequently display more pronounced cutin and wax-related absorption features when compared to shaded leaves (Ribeiro da Luz, 2006), and pea plants (*Pisum sativum* L.) (Liu *et al.*, 2019) exposed to higher temperatures showed the greatest variation in the main C-H stretching region (2975-2820cm^{-1}) associated with a majority of waxes, cutin and cutan (Heredia-Guerrero *et al.*, 2014; Guzmán-Delgado *et al.*, 2016) and variation in the carbonyl ester region (1750-1718cm^{-1}). With regards to humidity, a study on Northern Forest trees found high humidity shifted the metabolism profile in leaves to produce more non-structural carbohydrates, antioxidants and phenolic compounds (Oksanen *et al.*, 2018), as well as the altered chemical composition of the wax surface layer affecting the hydrophobic properties, and increasing the leaves susceptibility to fungal attack (Oksanen *et al.*, 2018). Low humidity can also

increase wax deposition: a study on *Brassica. oleracea*, *Eucalyptus gunni* and *Tropaeolum majus* found wax deposition to increase when relative humidity was reduced from 98% to around 20% (Koch *et al.*, 2006).

At the other end of whole-plant environment boundary, is the root-soil interface, whereby root anatomy can not only alter the diffusion distance across the root to the transporting vessels such as the xylem but also influence the exploratory power and metabolic costs of roots in the rhizosphere, thus affecting water and nutrient acquisition. Root anatomy is a key determinant of root water uptake and displays a great deal of phenotypic plasticity towards changing environmental conditions (Zhu *et al.*, 2011), to maximise uptake during optimal conditions, and elect efficient growth strategies when stressed (e.g. drought). The metabolic costs of soil exploration are very high and can exceed 50% of daily photosynthesis (Lambers, Atkin and Millenaar, 2002b), during resource deficient conditions, efficient root growth is key. Plants tend to increase root:shoot ratios during drought conditions (Fitter and Stickland, 1991), which results in each unit of leaf area supporting a higher proportion of non-photosynthetic tissue (Lynch, 2015). It is therefore beneficial for plants to opt for less metabolically demanding root tissues so that larger root systems which are more capable of resource acquisition can be maintained (Lynch, 2015).

This chapter aims to gain a better understanding of how maize and wheat respond to changes in soil moisture and humidity from a whole plant perspective, and how such changes may influence resource transport (water and nutrients) root growth efficiency, and exploratory power of the root system, by investigating changes to

various root anatomical features including aerenchyma, cortical cells, and the stele. Not much is known of the effects of low humidity and high VPD on root anatomy, even less is known about high humidity (low VPD). Low humidity (high VPD), has been observed to cause a reduction in endodermal cell size in pearl millet (Kholová *et al.*, 2016) as well as reduced stele diameters in wheat (Schoppach *et al.*, 2014). Most water-based studies focus on soil moisture status rather than atmospheric water status (humidity) when addressing root anatomical adaptations. An example of an adjustment in root structure and composition in response to adverse conditions is root cortical aerenchyma (RCA) formation, a product of programmed cell death (Schneider *et al.*, 2018)**.** Aerenchyma formation begins in the mid-cortex whereby gas spaces extend both radially and tangentially through the lysis of cells (Campbell and Drew, 1983), leaving spokes of connected cell walls (Lenochová, Soukup and Votrubová, 2009) separated by large cavities (Campbell and Drew, 1983). Such cavities can form under stress conditions such as hypoxia, high temperatures, drought and nutrient deficiency (Mohammed *et al.*, 2019). RCA is, therefore, a good indicator of plant stress and the prevailing environmental conditions that are causing such stresses, aerenchyma formation can affect water and oxygen transport.

During waterlogged conditions aerenchyma act as conduits transporting oxygen from the aerial organs to the hypoxic roots (Mohammed *et al.*, 2019). Conversely, amidst drought conditions, root cortical aerenchyma permits greater drought tolerance in maize through the reduced metabolic cost of soil exploration (Chimungu *et al.*, 2015), allowing greater root growth and water uptake (Zhu, Brown and Lynch, 2010). Furthermore, maize genotypes with high root cortical

aerenchyma formation, have been found to exhibit greater leaf water content, shoot biomass and grain yield, compared to low aerenchyma forming genotypes under drought conditions (Chimungu *et al.*, 2015). However, such effects may vary among species, as a drought study on rice (*Oryza sativa*) found aerenchyma formation decreased under increased drought conditions, having a minimal influence on water uptake (Henry *et al.*, 2012).

Another programmed cell death response, not too dissimilar from RCA is root cortical senescence (RCS). Both phenomena are influenced by ethylene production, affect the radial transport of nutrients (Schneider *et al.*, 2017), and root hydraulic conductivity (Fan *et al.*, 2007; Schneider *et al.*, 2017) as well as reducing the metabolic costs of soil exploration (Zhu, Brown and Lynch, 2010; Schneider *et al.*, 2017). However, despite similarities RCA and RCS represent two independent patterns of nuclear deletion in the cortex (Deacon, Drew and Darling, 1986). RCS involves the lysis of cells in the outer cortex, progressing inwards towards older tissue (Holden, 1975; Schneider *et al.*, 2017), leading to increased aliphatic suberin content in the endodermis (Schneider *et al.*, 2017). The heightened occurrence of RCS has been observed during times of edaphic stress such as nitrogen and phosphorous deficiency (Schneider *et al.*, 2017) and appears to be limited to temperate monocots such as Barley (*Hordeum vulgare*), wheat (*Triticum aestivum*), triticale (*Triticosecale*) (Yeates and Parker, 1986; Liljeroth, 1995), rye (*Secale cereal*) (Deacon and Mitchell, 1985; Jupp and Newman, 1987) and oat (*Avena sativa*) (Yeates and Parker, 1986), unlike RCA which occurs in many species including those which also undergo RCS such as wheat and barley (Schneider *et al.*, 2018).

Both RCA and RCS can influence physiological processes which are critical to plant functioning such as hydraulic conductivity, radial movement of nutrients, and metabolic costs of soil exploration (Fan *et al.*, 2007; Zhu, Brown and Lynch, 2010; Schneider *et al.*, 2017, 2018). It is therefore important to investigate how above and belowground stresses may affect their occurrence and subsequent impacts on whole plant physiology. However, programmed cell death in the cortex through RCA and RCS are not the only mechanisms in which plants can influence processes such as water uptake and soil exploration, direct changes to cortical cell size and density can also play a significant role.

When a plant is growing in sub-optimal conditions, for example, low soil water availability, efficient growth is key. Larger diameter cortical cells are favoured for their reduced metabolic costs for growth (Colombi *et al.*, 2019), aiding exploration for scarce resources in a more efficient manner. In addition, reducing cortical cell file number (CCFN) improves drought tolerance by also reducing the metabolic costs of soil exploration (Chimungu, Brown and Lynch, 2014). A study on maize also found that genotypes with lower CCFN, exhibited significantly higher rates of stomatal conductance, greater leaf carbon assimilation and higher shoot biomass, compared to genotypes with many cortical cell files (Chimungu, Brown and Lynch, 2014). A change in cortex morphology, therefore, can significantly impact the whole plant, both above and belowground. We can also expect changes to CCFN to affect root hydraulic conductivity, as lower CCFN will reduce the distance that water needs to travel across the root before reaching the stele, ready for transport to the rest of the plant. Finer roots and those with a

thinner cortex are generally associated as maintaining higher root hydraulic conductivities (Rieger and Litvin, 1999).

The final mechanisms of anatomical adaptation to water deficit conditions we will be investigating are changes within the stele. The stele contains major transport vessels (xylem and phloem) for both water and nutrient transport throughout the plant. It is encompassed by an endodermis layer that separates the inner vascular tissues from the surrounding cortex, acting as an apoplastic barrier and facilitating selective nutrient uptake (Miyashima and Nakajima, 2011). Consequently, changes to stele anatomy will also influence root hydraulic conductivity, metabolic costs of root growth, and root penetrability through the soil column. With regards to soil exploration, increased stele diameters are associated with reduced root tensile strengths (Chimungu, Loades and Lynch, 2015) and are therefore less well equipped for root penetration in hard soils. As soil strength increases nonlinearly with decreasing soil moisture, dry soils may considerably limit plant growth in terms of not only water availability for the plant but also mechanical impedance (Chimungu, Loades and Lynch, 2015). Reduced stele diameters could also be beneficial during low humidity (high VPD) conditions, by limiting the rate of transpiration sensitivity to changing VPD conditions, a study on drought-tolerant wheat lines (*Triticum aestivum* RAC875) showed significantly smaller stele diameters during high VPD conditions (Schoppach *et al.*, 2014). Though this could be considered a water-saving strategy during high VPD conditions, during low soil moisture, the opposite response was observed in rice. During drought conditions, a study on rice grown at relatively high humidity (66-71%) found increases in stele diameter as a percentage of total root diameter,

implying that the plant is favouring water retention (Henry *et al.*, 2012), during soil water limiting conditions.

Responses to water deficit condition are not just dependent on the size of the stele, but also the vessels that lie within. Xylem vessels contained in the stele are specialised tissues that facilitate the movement of water and nutrients from roots to the aerial organs, as well as providing storage and mechanical support (Myburg, Lev-Yadun and Sederoff, 2013). If xylem water potentials drop below the xylem-specific threshold air can enter the conduit from adjacent air-filled cells, interrupting capillary action (Brodribb, McAdam and Carins Murphy, 2017) and leading to cavitation (Zimmermann, 2013). The risk of cavitation is higher during drought conditions due to reduced soil water supply and low stem water potentials. Survival during these conditions depends on a plants ability to respond and reduce the risk of cavitation and subsequent operational failure of xylem vessels (Sperry and Sullivan, 1992). By reducing the number of xylem vessels (Henry *et al.*, 2012) and/or size (Fichot *et al.*, 2009), reduces the area available for cavitation to occur and concentrates transpiration streams. Plants that can lower their hydraulic requirements are considered to be more water-use efficient and better equipped to cope with drought stress conditions (Fichot *et al.*, 2009) and reduced metaxylem size in wheat has been linked to increased yields during low soil moisture conditions (Richards and Passioura, 1989). Conversely, during well-watered conditions plants can reduce resistance to water movement from the soil to the root by increasing xylem diameters (Wasson *et al.*, 2012). As such, any changes to the root's cortex or stele in response to prevailing environmental conditions will influence the whole root diameter, as it is a function of both stele

area and cortical cell thickness (Chimungu, Loades and Lynch, 2015). During water limiting conditions, plants can concentrate resources by reducing their root cross-sectional area (Poorter and Remkes, 1990; Henry *et al.*, 2012) and lowering the metabolic costs of soil exploration (Sharp *et al.*, 2004; Hund, Ruta and Liedgens, 2009; Lynch, 2013), subsequently reducing the distance substances need to travel from soil to plant.

The above summarises how cuticle chemistry and root anatomy can influence water movement, but their properties are responsive to environmental conditions. They lie at very different ends of the plant system but remain very much connected. Previous studies have highlighted links between aerial and subterranean plant organs, with a positive relationship between relative leaf area and rooting depth (Sadras *et al.*, 1989). Also, plants with high-density roots are associated with high-density leaves and low-density roots with low-density leaves (Craine *et al.*, 2001) and more recently, high stomatal densities have been affiliated with larger root areas (Hepworth, *et al*., 2016). Such findings suggest the presence of a coordinated strategy between the aerial and subterranean organs, that is finely tuned to changes in environmental conditions, both above and below the ground.

This study will investigate the potential changes to both leaf cuticle chemistry and root anatomy, in response to changing humidity and soil moisture conditions. Through examining such responses at the leaf-atmosphere interface and the root-soil interface, we begin to understand how a plant can influence diffusion

distances and alter subsequent resistance to water loss/gain under different environmental conditions.

4.2 AIMS AND OBJECTIVES

The main aims of this chapter are to identify any spectral chemical changes to leaf cuticles as well as any changes to root anatomy in response to treatment conditions (humidity and/or soil moisture content).

Hypotheses

1. Low soil moisture will promote root cortical aerenchyma (RCA) formation in maize, regardless of humidity.
2. Low soil moisture will promote root cortical senescence (RCS) in wheat regardless of humidity.
3. Low humidity (high VPD) will cause a reduction in stele area, regardless of soil moisture content.
4. There will be no major differences between abaxial and adaxial cuticle chemistry within both maize and wheat.
5. Humidity will cause alterations in cuticle chemistry which relate to wax deposition regardless of soil moisture content.

4.3 MATERIALS AND METHODS

4.3.1 Plant Material and Experimental Design

Initial germination and growth conditions of plants echo methods from Chapter 2 and Chapter 3. 16 maize (*Zea mays* cv.) and 16 wheat (*Triticum aestivum* cv. Paragon) seeds were germinated in 2L pots, packed to a bulk density of 1.3 g cm^{-3}, with a sandy loam collected from a field site in Bunny, Leicestershire (Longitude=-1.12608866, Latitude=52.86098725). During germination, soil moisture for both treatments was maintained at 70% field capacity.

Four reps of maize and wheat were randomly arranged in the growth chamber subjected to the following treatment conditions (Table 1). Soil moisture was maintained with regular weighing and watering. Air temperature and humidity were recorded every hour in either side of the growth chamber (high and low humidity compartments), using a Fisher Scientific Traceable Humidity/Temperature. Dew-Point Meter (Fisher, UK), recorded values are presented in Figure 1 (daily averages) and Figure 2 (day and night averages). Vapour pressure deficit (VPD) values were calculated using recorded air temperature and humidity data using methods and equation detailed in Chapter 2 methodology (2.3).

Table 1. Treatment growth conditions. Relative humidity and temperature measurements were recorded using a Fisher Scientific Traceable Humidity/Temperature. Dew-Point Meter (Fisher, UK). Day refers to 06:00 – 18:00 and night (18:01 – 05:59). Soil moisture treatment was maintained with regular watering to weight.

Treatment	**Relative Humidity (%) day/night**	**Soil Moisture (field capacity %)**	**Temperature °C (day/night)**	**Calculated Air Vapour Pressure Deficit (VPD_{air} kPa) (day/night)**
High Humidity High Soil Moisture **(HHHS)**	94.63/99.72	70	22.58/15.92	0.28/0.004
High Humidity Low Soil Moisture **(HHLS)**	94.63/99.72	30	22.58/15.92	0.28/0.004
Low Humidity High Soil Moisture **(LHHS)**	32.85/47.51	70	22.52/15.71	2.03/0.95
Low Humidity Low Soil Moisture **(LHLS)**	32.85/47.51	30	22.52/15.71	2.03/0.95

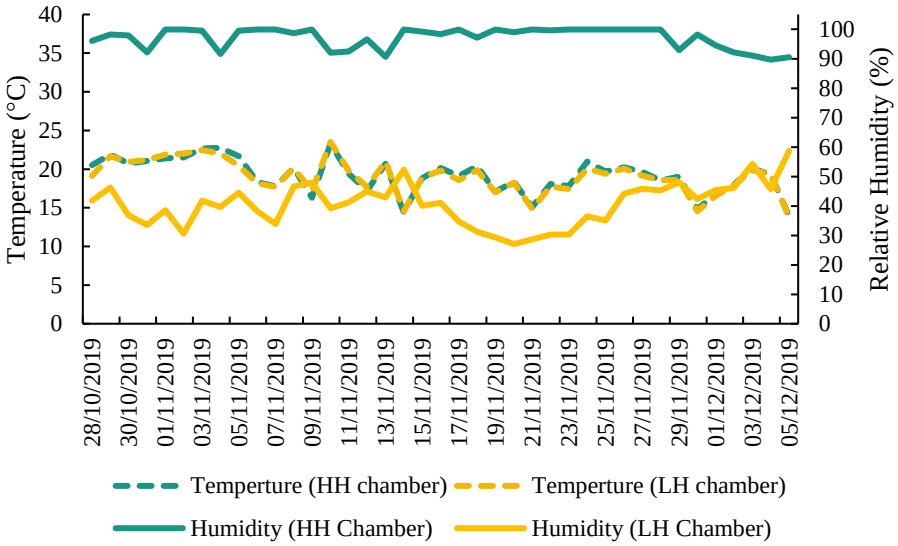


Figure 1. Daily averages of the growth conditions in the high humidity (■) and low humidity (■) chambers, throughout the experiment. Measurements recorded using a Fisher Scientific Traceable Humidity/Temperature. Dew-Point Meter (Fisher, UK).

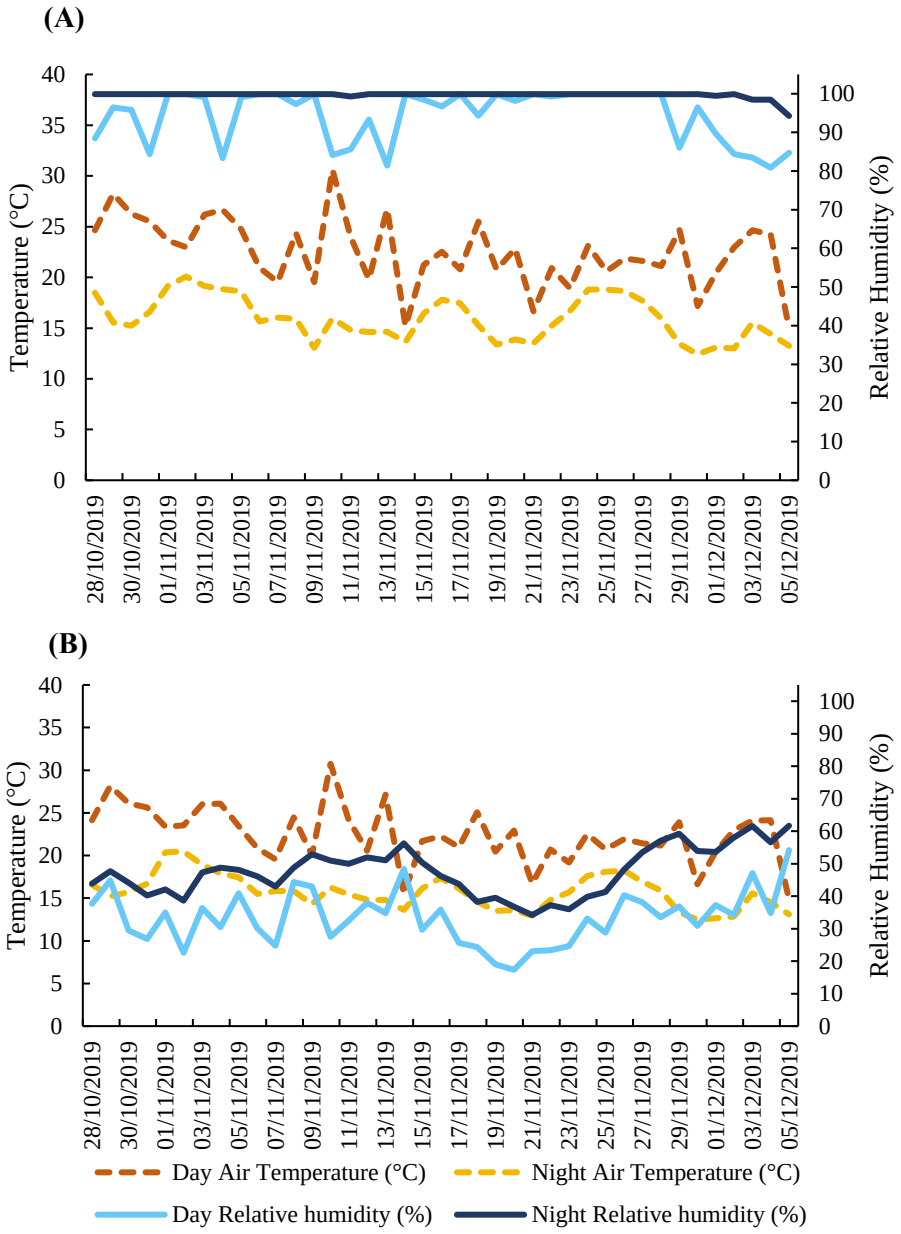


Figure 2. Day and night average temperature and relative humidity in the high humidity growth chamber (A) and the low humidity chamber (B). Measurements were recorded using a Fisher Scientific Traceable Humidity/Temperature. Dew-Point Meter (Fisher, UK). Day refers to 06:00 – 18:00 and night 18:01 – 05:59.

4.3.2 Root Anatomy

Maize and wheat were gently removed from the 2L pots, where roots were washed as thoroughly as possible whilst attempting to minimise root handling

which would lead to damaged samples. Maize and wheat seminal root sections were collected from three-week-old plants, two sections (3cm and 13cm away from the root tip) per root were cut with a razor blade. All root samples collected were 3.5-4cm in length and placed in a 50ml falcon tube filled with tap water to prevent desiccation and damage from overhandling. The samples were stored in the falcon tubes at room temperature for no more than 2 hours, whilst embedding procedures were set up.

4.3.2.1 Embedding

Materials and methods were adapted from the paper by (Atkinson and Wells, 2017). Briefly, the methods were as follows. Root samples were placed into custom-designed, 3D printed polylactic acid (PLA) moulds (as described in (Atkinson and Wells, 2017)). At least a 4mm gap was left between the samples, with approximately four root sections fitting on each mould.

5% (w/v) agarose (Sigma-Aldrich, Co. Ltd) was prepared before root cutting and stored in a 55°C incubator until use. The moulds were then filled with agarose once it had cooled to 39°C, then the samples were left for the gel to set.

4.3.2.2 Sectioning

Sections were taken using a vibrating microtome (7000smz-2, Campden Instruments Ltd). Sectioning settings are displayed in Table 2.

Table 2. Vibrating microtome (7000smz-2, Campden Instruments Ltd) settings for maize and wheat seminal roots.

Root type	**Section thickness (µm)**	**Blade speed (mm/s)**	**Blade frequency (Hz)**
Maize seminal	250	0.57-0.82	55-65
Wheat seminal	250	0.57-0.82	65

4.3.2.3 Staining

After sectioning, root sections were removed from the vibratome bath and incubated in a calcofluor white (Sigma-Aldrich, Co. Ltd) solution (0.3mg/ml) for approximately 60 seconds. Sections were then rinsed with deionised water and placed on a microscope slide.

4.3.2.4 Image acquisition

The maize and wheat sections were then observed using an Eclipse Ti CLSM confocal laser scanning microscope (Nikon Instruments) at ×10 (maize) and ×20 (wheat) objectives. Three image channels were collected per cross-section image (red, blue, and green) see Table 3 for further confocal laser scanning microscope settings.

Table 3. Confocal laser scanning microscope settings used to produce the multicolour images for analysis. Gain varied slightly between each sample to achieve the clearest and brightest image without allowing for too much background noise.

Image channel	**Laser (nm)**	**Filter**	**Gain**	**Pinhole size**	**Tissue**
1	408	450/35 (blue)	~65	Medium	Phloem
2	408	515/30 (green)	~120	Small	All cell walls
3	488	605/75 (red)	~100	Small	Xylem vessels, epidermis, and secondary thickening.

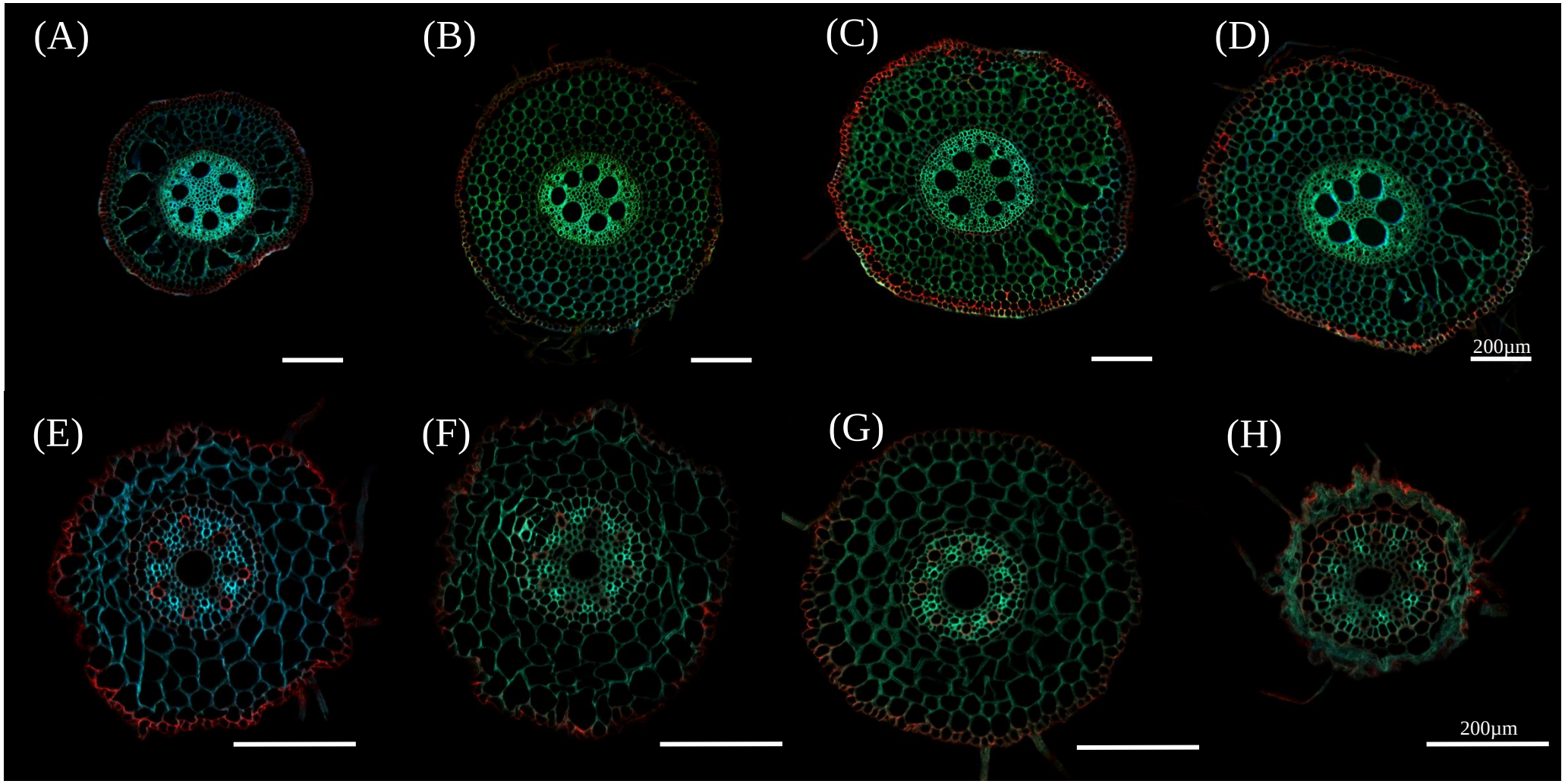


Figure 3. Cross-section images of maize, top row (A,B,C,D) and wheat, bottom row (E,F,G,H) seminal roots collected 13cm from the root tip. Roots from the four treatments are presented: High Humidity High Soil Moisture (A and E), High Humidity Low Soil Moisture (B and F), Low humidity High Soil Moisture (C and G) and Low Humidity Low Soil Moisture (D and H). Objectives used ×10 for maize and ×20 for wheat. Scale bar represents 200µm.

4.3.2.5 Image analysis

Individual channels were combined in a composite image in open source image software Fiji. Images used in figures were further processed in Adobe Photoshop whereby contrast, exposure and highlights were adjusted for optimal viewing quality. Root cross-section images were analysed and measured in Fiji (ImageJ). The number of metaxylem, xylem, phloem and cortical cells were counted using the plugin 'cell counter'. Using cell counter, 10 observations of cortical cell file number were recorded and averaged. The stele area and whole root area were calculated from the radius of each respectively and the cortical cell area was calculated as:

$$whole\ root\ area - stele\ area = cortical\ cell\ area$$

The area of aerenchyma (when present) was measured using the freehand tool in Fiji (ImageJ), whereby an outline was drawn around the aerenchyma and area automatically calculated.

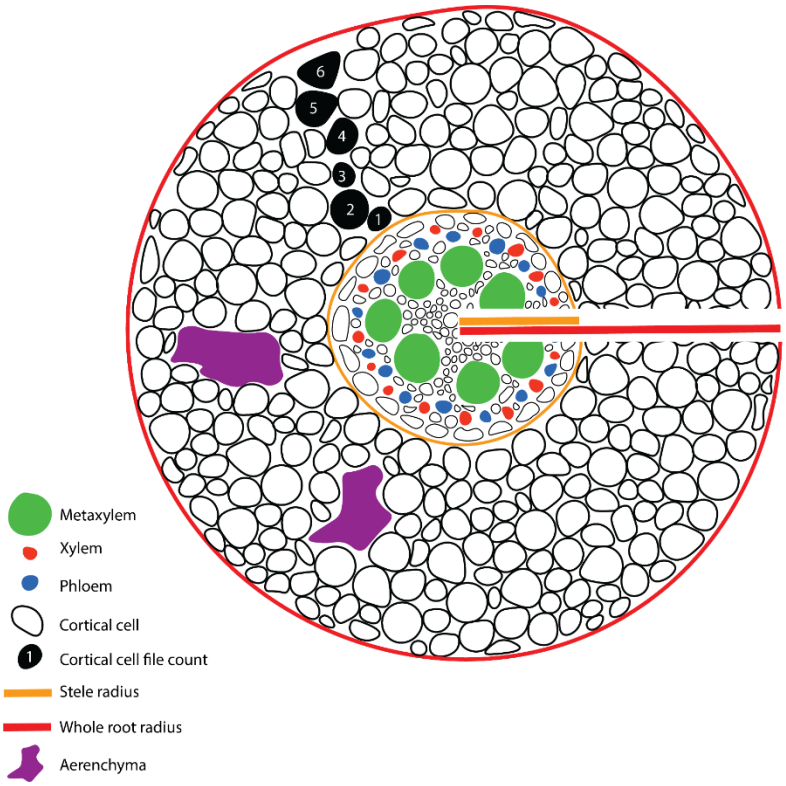


Figure 4. Cross-sectional schematic representing the areas of the root that were measured and counted. The number of metaxylem, xylem, phloem, cortical cells, and the number of cortical cell files were counted using Fiji (ImageJ) plugin 'Cell counter'. Stele and whole root cross-section area were calculated from the radius of each, respectively. The area of the aerenchyma (when present) was measured using the freehand measure tool in ImageJ where an outline was drawn, and the area automatically calculated.

4.3.3 Leaf Cuticle Chemistry

Methods follow a similar protocol set out in (Jardine *et al.*, 2019), using attenuated total reflectance Fourier Transform infrared (ARE-FTIR) spectroscopy to analyse leaf cuticles from maize and wheat plants grown across the four treatments.

4.3.3.1 Sample collection

For the FTIR analyses, data were generated from three leaf samples (~1 × 1 cm) exercised from the longest unfurled leaf. Samples were collected at even intervals along the leaf, close to the tip, middle and base of the leaf. The samples were stored in falcon tubes, whilst awaiting FTIR analysis carried out the next day.

The IR spectra were generated using a Cary 670 FTIR spectrometer combined with a Cary 620 FTIR microscope (Agilent, Santa Clara, CA, USA). The FTIR microscope was equipped with a 64×64 pixel focal plane array (FPA) detector and a 15× Vis/IR objective at high magnification to which a Germanium crystal micro-attenuated total reflectance (ATR) was fitted. This set up achieved a resolution of 1.1μm per pixel (each pixel results in one IR spectrum, therefore each measurement produces an array of 64×64=4096 spectra). One abaxial and one adaxial measurement per sample placed in direct contact with the crystal were collected at 64 scans per measurement and a resolution of 4. Background spectra were collected before each set of replicates and automatically removed from the sample spectra.

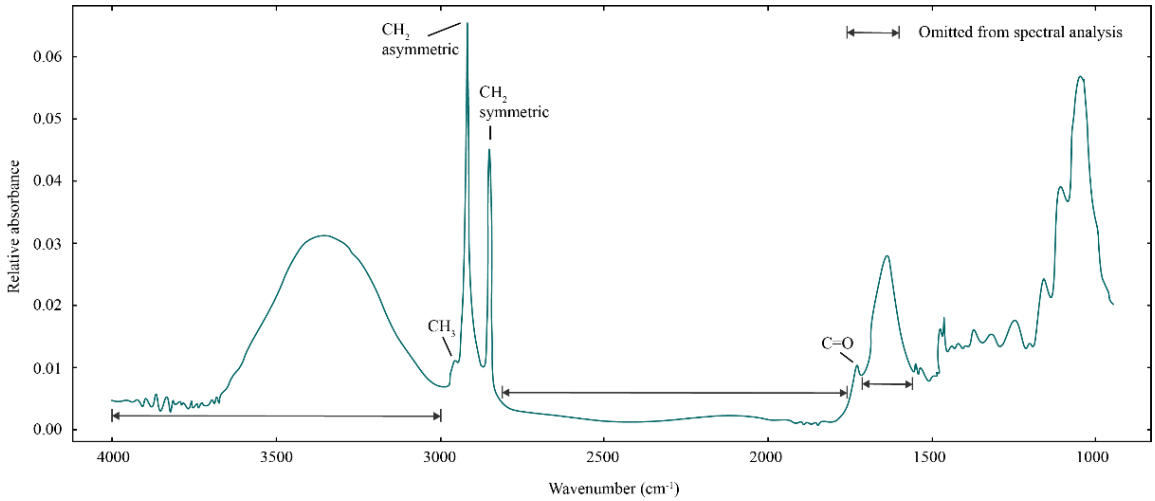


Figure 5. Typical ATR-FTIR spectrum of maize (*Zea mays*) cuticle from ATR-FTIR. Wavelengths omitted from the spectral analysis are highlighted, along with waveband peaks of interest that are associated with cuticle waxes.

4.3.3.2 Data Analyses

Spectral differences across the four treatment conditions were analysed by Principal Components Analysis (PCA), t-Distributed Stochastic Neighbour Embedding (t-SNE), and Chi^2 test of the highest absorbances of selected peaks of interest (wavebands associated with cuticle waxes). Due to the large volume of compositional data retrieved from ATR-FTIR spectroscopy, unsupervised machine learning and dimensionality reduction techniques such as PCA and t-SNE are useful data analysis tools that allow us to discover trends in high-dimensional data that would otherwise be very challenging to observe.

As fresh leaf samples were scanned, the broad peak around 3400 cm^{-1} and peaks around 1650 cm^{-1} were excluded from the multivariate analysis of the data, due to the potential interference of OH- from water dominating the multivariate analysis due to the variability in water content of the leaves (Liu *et al.*, 2019). As such. the following regions were excluded from the spectroscopic analysis: 1590 to

1700cm^{-1} and wavelength above 3000cm^{-1}. Furthermore, the region from 1600 cm^{-1} to 2800 cm^{-1} was also omitted from the spectroscopic analysis, between the aliphatic peaks and the fingerprint region where there were minimal spectral absorbance and relevant chemical information with regards to scanning a plant cuticle. The fingerprint region (~1500-500 cm^{-1}) is a region of the spectrum that is almost unique to any given compound (like the human fingerprint).

Pre-processing of the compositional data was carried out using an Extended Multiplicative Scatter Correction (EMSC) baselining method, with 2nd order polynomial and derivative smoothing. The potential spectral differences caused by treatment effects (both humidity and soil moisture) and also any spectral differences between the abaxial and adaxial sides of the leaf were firstly explored with Principal Components Analysis (PCA), followed by a t-distributed stochastic neighbour embedding (t-SNE) algorithm (Van Der Maaten and Hinton, 2008), to explore and visualise potential clustering in the data. A t-SNE Is one of the most powerful dimensionality reduction techniques and was chosen to be carried out on this data due to the first few components of the PCA explaining little variance (Platzer, 2013), (data not presented). The t-SNE is more appropriate for high dimensionality data (as observed in this chapter), and more capable at revealing local structure in the data, resulting in clustering of data points.(Kobak and Berens, 2019), therefore only t-SNE plots are presented in this chapter.

All statistical analyses for root anatomy and Chi^2 comparing peaks in the leaf spectral data were carried out in Genstat 20th Edition. PCA and t-SNE were carried out in R.

4.4 RESULTS

4.4.1 Root Anatomy

4.4.1.1 Maize

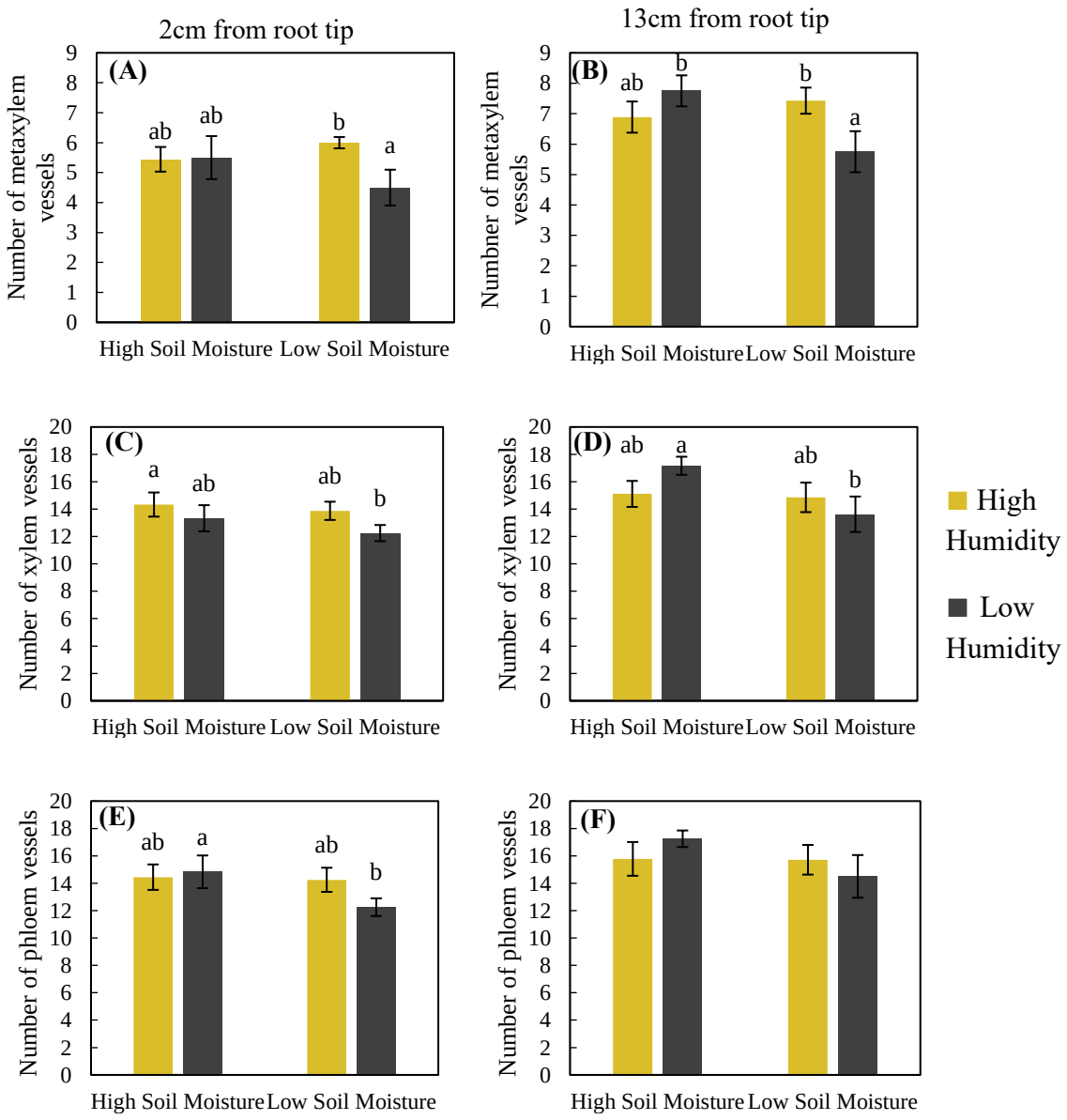


Figure 6. The effects of the four treatments High Humidity High Soil moisture, High Humidity Low Soil moisture, Low Humidity High Soil moisture and Low Humidity Low Soil moisture on maize root anatomy three weeks after germination. Roots were sampled 2cm from the tip (panels A, C, E) and 13cm from the tip (B, D, F). Panels number of metaxylem vessels at 2cm (A) and 13cm (B), number of xylem vessels at 2cm (C) and 13cm (D), and number of phloem vessels at 2cm (E) and 13cm (F). Error bars represent ±

SE and different letters represent significant differences at the 5% level after a post-hoc Tukey test. n=4)

The number of maize metaxylem vessels, at 2cm (Figure 6A) and 13cm (Figure 6B) were significantly affected by a humidity soil moisture interaction (P=0.027 and P=0.004 respectively. Whereby, high humidity increased the number of metaxylem vessels when soil moisture is low. The number of maize xylem vessels was significantly higher in high humidity (P=0.022) at 2cm (Figure 6C). Whereas at 13cm (Figure 6D) soil moisture had a significant main effect (P=0.01), as well as a significant interaction between humidity and soil moisture (P=0.026) whereby under low humidity conditions, high soil moisture resulted in significantly more xylem vessels. Significant differences in maize phloem vessel number were only detected in tissues measured at 2cm (Figure 6E), with soil moisture having a significant effect (P=0.031) and an interaction between close to significance at the 5% level (P=0.065). Under low humidity conditions, low soil moisture resulted in fewer phloem vessels.

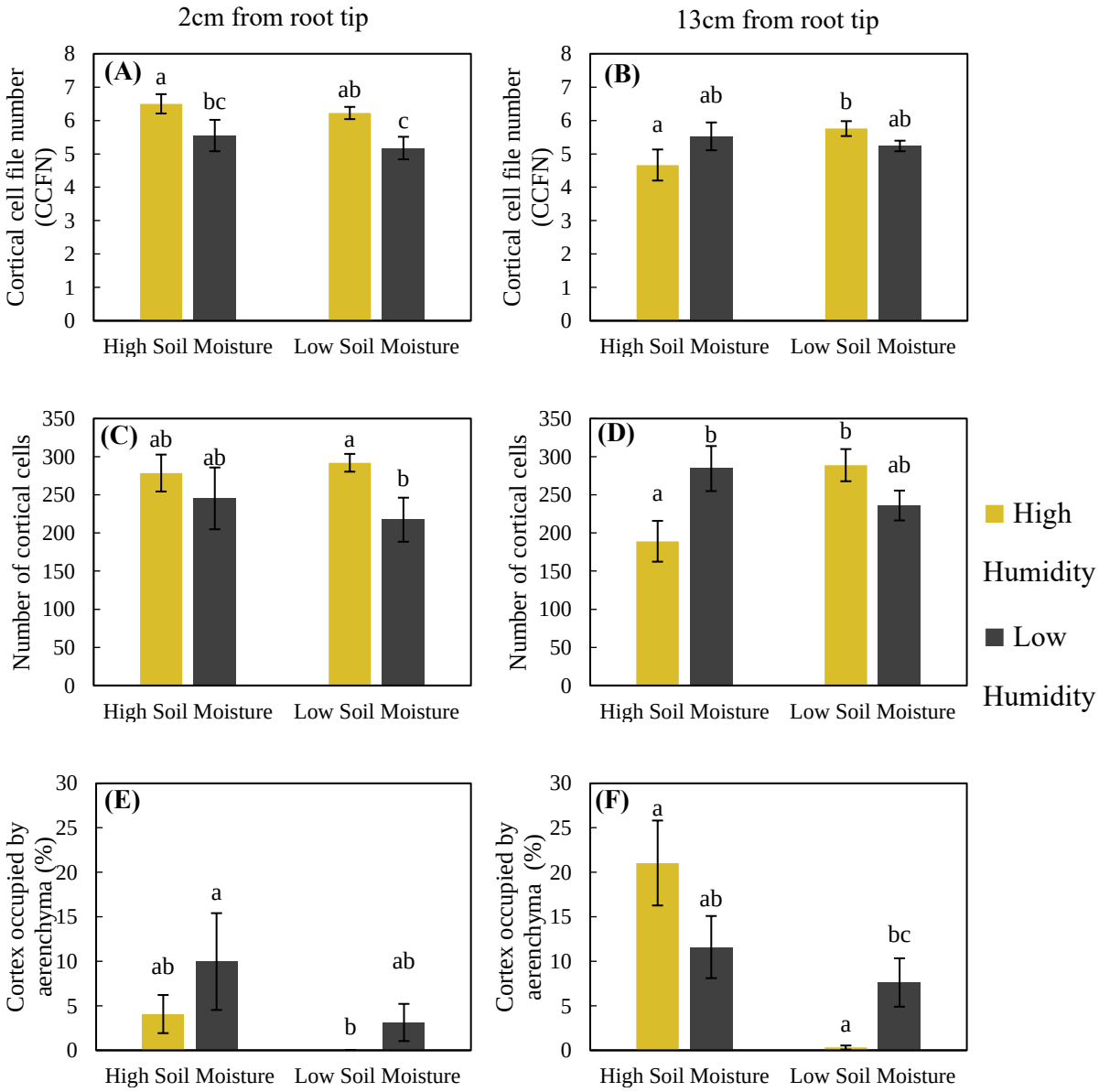


Figure 7. The effects of the four treatments High Humidity High Soil moisture, High Humidity Low Soil moisture, Low Humidity High Soil moisture and Low Humidity Low Soil moisture on maize root anatomy three weeks after germination. Roots were sampled 2cm from the tip (panels A, C, E) and 13cm from the tip (B, D, F). Panels display cortical cell file number at 2cm (A) and 13cm (B), cortical cell count at 2cm (C) and 13cm (D) and percentage of cortex occupied by aerenchyma at 2cm (E) and 13cm (F). Error bars

represent ± SE and different letters represent significant differences at the 5% level after a post-hoc Tukey test. n=4

There was limited age-related variation between younger and older maize root segments in response to humidity and soil moisture treatments. Cortical cell file number measured on younger maize tissue at 2cm (Figure 7A) was significantly higher at high humidity ($P<0.001$), whereas older tissues measured at 13cm (Figure 7B) were significantly affected by a humidity soil moisture interactions ($P=0.02$) whereby under high humidity conditions, low soil moisture resulted in significantly more cortical cell files. Similar results were recorded in terms of cortical cell number, as high humidity led to significantly more maize cortical cells ($P=0.007$) in 2cm sampled tissues (Figure 7C). Whereas at 13cm (Figure 7D) the number of maize cortical cells was affected by a humidity soil moisture interaction ($P<0.001$), under high soil moisture conditions, high humidity led to significantly fewer cortical cells, and under high humidity conditions, low soil moisture resulted in significantly more cortical cells.

The percentage of maize cortex occupied by aerenchyma at 2cm (Figure 7E) was significantly affected by humidity and soil moisture as main effects ($P=0.018$ and $P=0.005$ respectively), as low soil moisture and low humidity resulted in fewer aerenchyma formations. At 13cm (Figure 7F), in comparatively older tissue, the percentage of cortex occupied by aerenchyma was significantly lower in low soil moisture conditions ($P<0.001$) and was also affected by a humidity soil moisture interaction ($P=0.003$) whereby high humidity increased aerenchyma formation in high soil moisture conditions, but reduced aerenchyma formation under low soil moisture conditions.

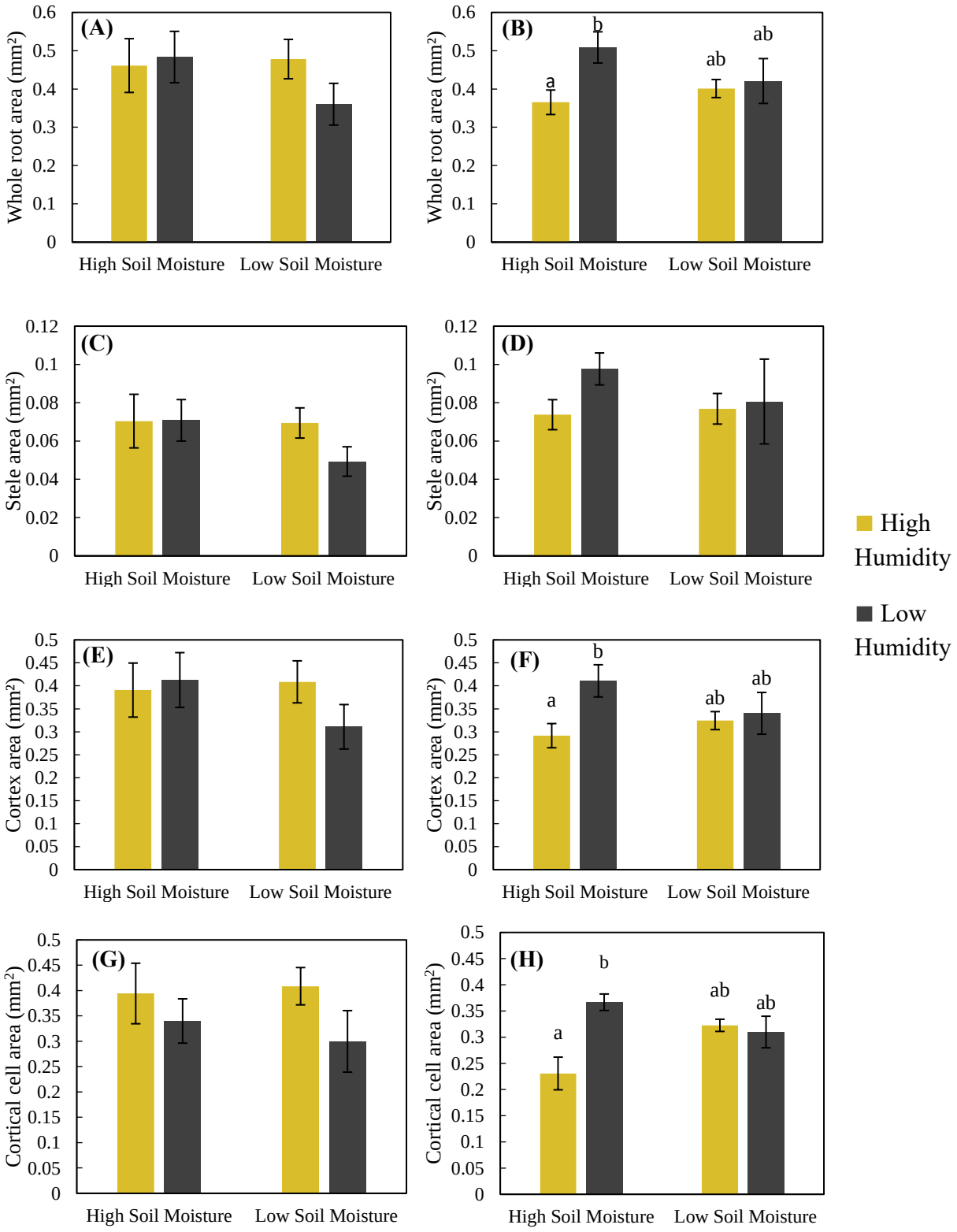


Figure 8. The effects of the four treatments High Humidity High Soil moisture, High Humidity Low Soil moisture, Low Humidity High Soil moisture and Low Humidity Low Soil moisture on maize root anatomy, three weeks after germination. Roots were sampled

2cm from the tip (panels A,C,E,G) and 13cm from the tip (B, D, F,H). Panels display whole root cross-sectional area at 2cm (A) and 13cm (B), stele area at 2cm (C) and 13cm (D), cortex area at 2cm (E) and 13cm (F), cortical cell area at 2cm (G) and 13cm, displayed as mm². Error bars represent ± SE and different letters represent significant differences at the 5% level after a post-hoc Tukey test. n=4

Root segments analysed 13cm away from the tip showed maize whole root area (Figure 8B), cortex area (Figure 8F), and cortical cell area (Figure 8H) were significantly affected by both humidity as a main effect (P=0.009, P=0.008, and P=0.044 respectively) and a significant interaction between humidity and soil moisture (whole root area and cortex area P=0.05, cortical cell area P=0.019). During high soil moisture conditions, high humidity led to significantly smaller whole root area, cortex area, and cortical cell area.

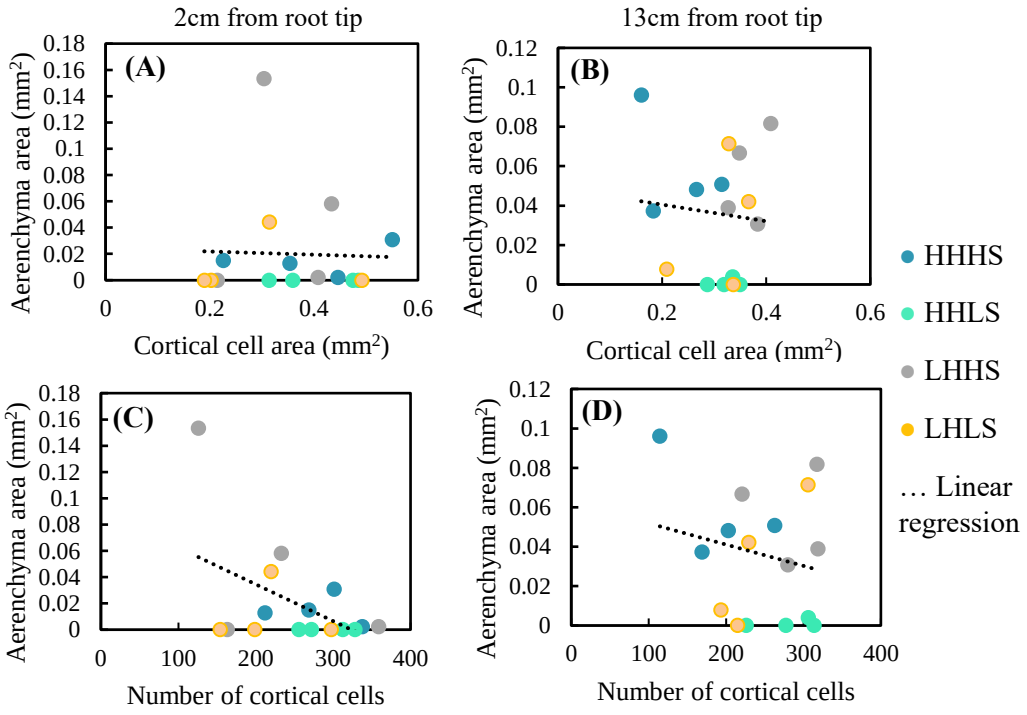


Figure 9. Scatter plots showing lack of significant correlations between maize cortical cell area and aerenchyma area measured at 2cm (A) and 12cm (B) away from the root tip and comparing number of cortical cells and aerenchyma area measured at 2cm (C) and 12cm

(D) away from the root tip. Linear regressions are based on all data points, each treatment n=4.

There were no significant correlations between aerenchyma area and the cortex (both cortical cell area and number of cortical cells) (Figure 9). Linear regressions are based on all data points, each treatment n=4, R^2 values for (A) 0.0012, (B) 0.0086, (C) 0.2384, and (D) 0.0417.

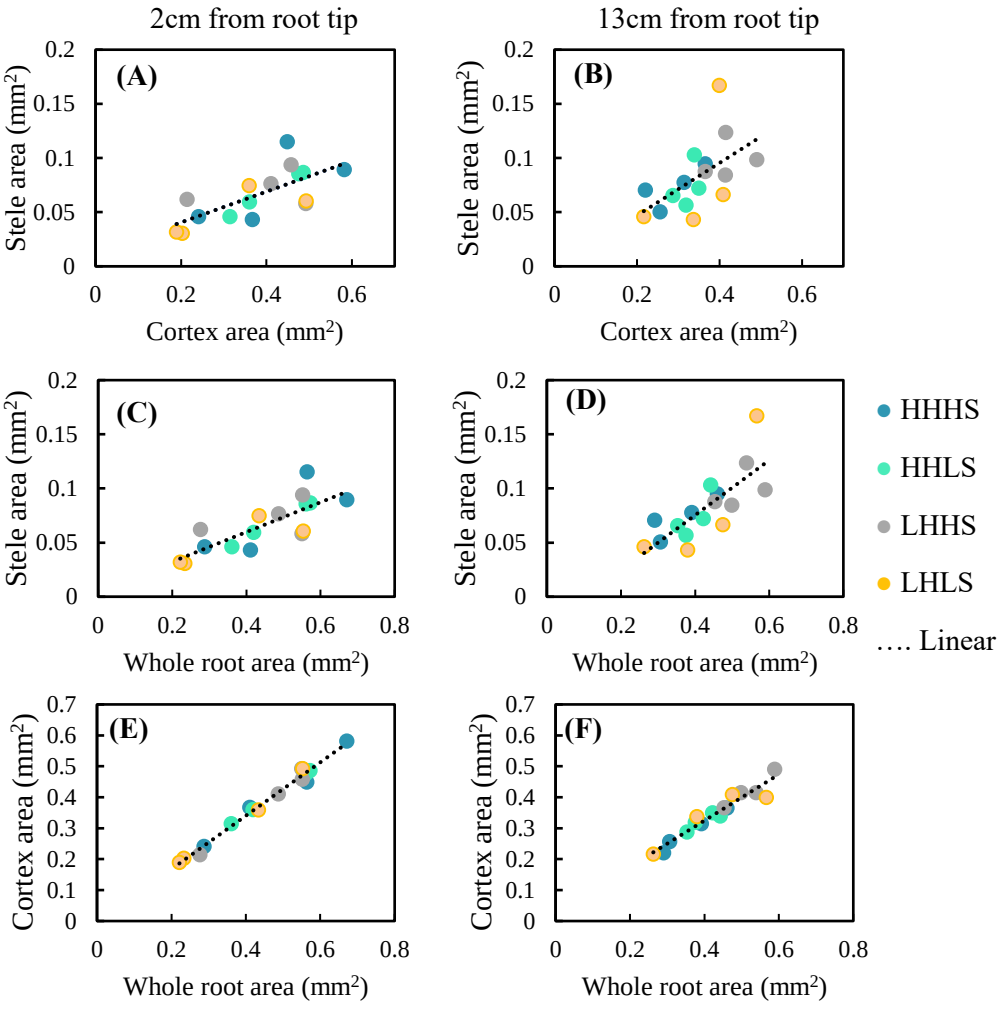


Figure 10. Scatter plots displaying correlations between maize cortex area and stele area at 2cm (A) and 13cm (B) from the root tip, whole root area and stele area at 2cm (C) and

13cm (D), and whole root area and stele area at 2cm (E) and 13cm (F). Dotted line represents linear regression based on all data points, each treatment n=4.

There were multiple positive correlations (Figure 10) between root anatomy traits represented by a dotted line through the data points, r^2 values are (A) 0.5086, (B) 0.3263, (C) 0.6288, (D) 0.5953, (E) 0.983, and (F) 0.9271.

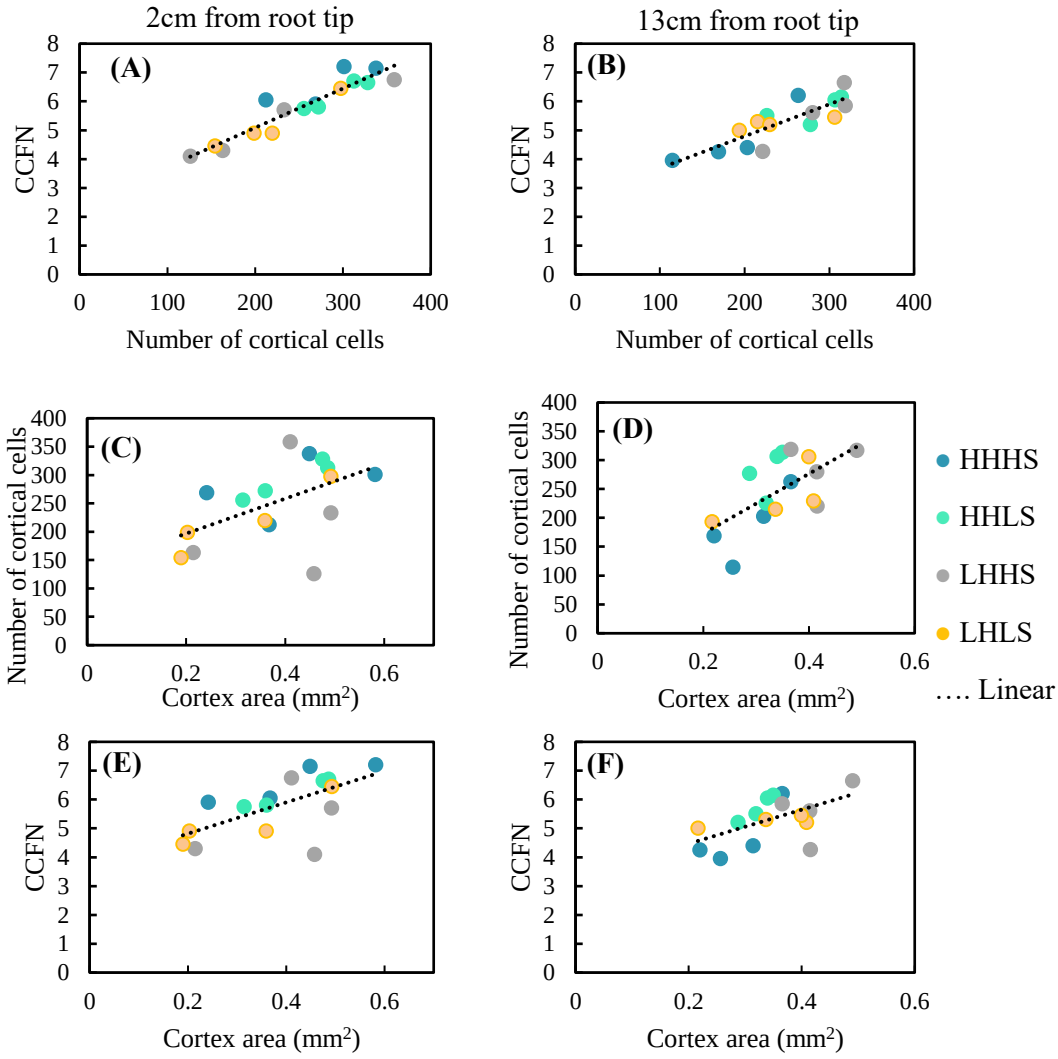


Figure 11. Scatter plots comparing maize number of cortical cells and cortical cell file number (CCFN) at 2cm (A) and 13cm (B) from the root tip, cortex area and number of cortical cells at 2cm (C) and 13cm (D), and cortex area and CCFN at 2cm (E) and 13cm (F). Dotted line represents linear regression based on all data points, each treatment n=4, R^2 values (A) 0.8732, (B) 0.7248, (C) 0.284, (D) 0.4106, (E) 0.418, and (F) 0.3139.

There is a strong positive correlation between cortical cell file number (CCFN) and number of cortical cells measured (Figure 11) at 2cm from the maize root tip (r^2=0.87) and 13cm from the root tip (r^2=0.74).

4.4.1.2 Wheat

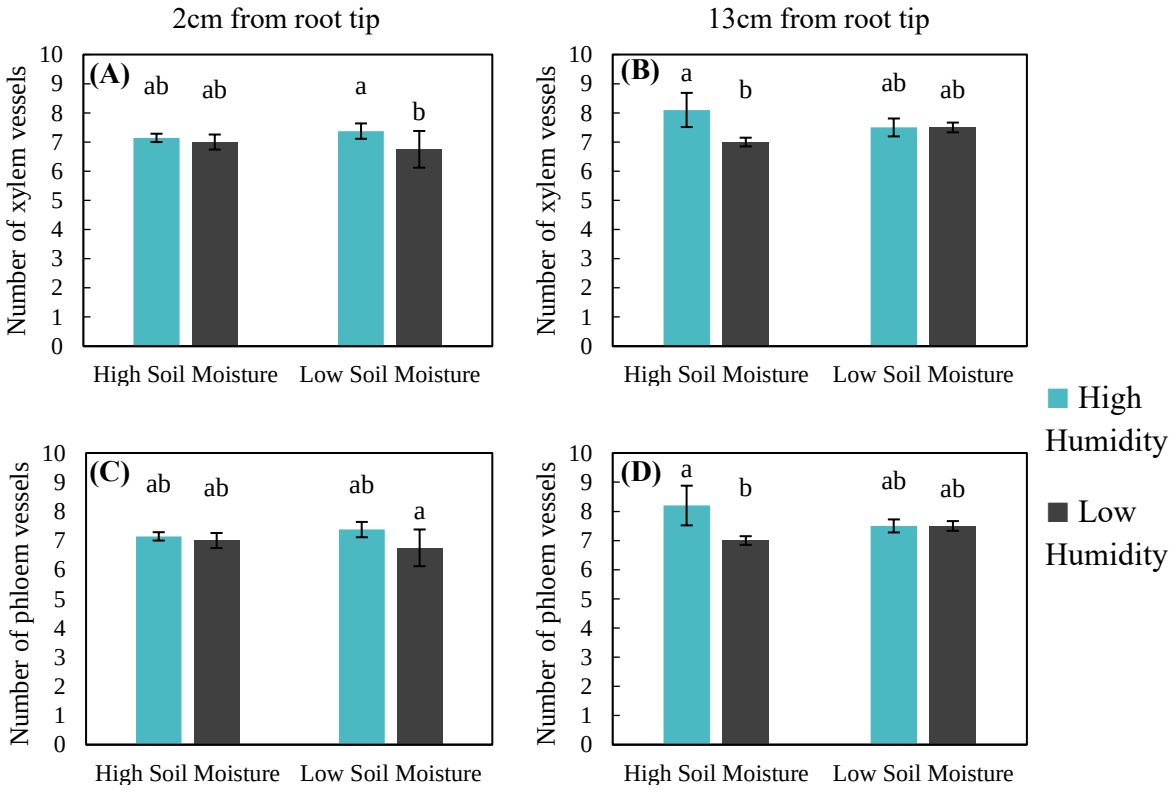


Figure 12. The effects of the four treatments High Humidity High Soil moisture, High Humidity Low Soil moisture, Low Humidity High Soil moisture and Low Humidity Low Soil moisture on wheat plants three weeks after germination. Roots were sampled 2cm from the tip (panels A and C) and 13cm from the tip (B and D). Panels display number of xylem vessels at 2cm (A) and 13cm (B), and the number of phloem vessels at 2cm (C) and 13cm (D). Error bars represent ± SE and different letters represent significant

differences at the 5% level after a post-hoc Fisher's unprotected least significant difference test n=4.

The number of wheat xylem vessels measured 2cm away from the tip (Figure 12A) and 13cm (Figure 12B) was significantly affected by humidity as a main effect (P=0.023 and P=0.042 respectively) as high humidity led to more xylem vessels. At 13cm (Figure 12B) there was also a significant interaction between humidity and soil moisture (P=0.045), whereby under high soil moisture conditions, high humidity resulted in significantly greater numbers of xylem vessels. Similar findings were reported for wheat phloem vessels measured 2cm from the root tip (Figure 12C) and 13cm (Figure 12D), as high humidity led to significantly more phloem vessels (P=0.023 and P=0.039 respectively) also, at 13cm there was a significant interaction between humidity and soil moisture (P=0.041), under high soil moisture conditions, high humidity led to significantly more wheat phloem vessels.

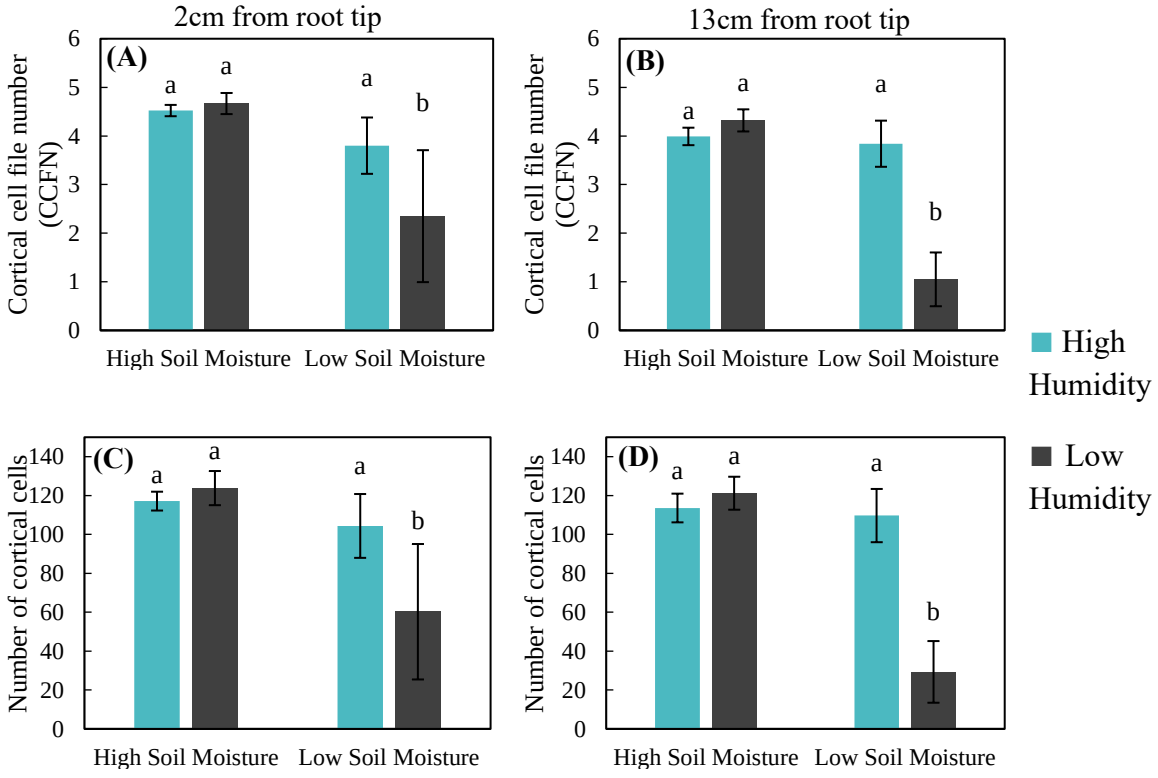


Figure 13. The effects of the four treatments High Humidity High Soil moisture, High Humidity Low Soil moisture, Low Humidity High Soil moisture and Low Humidity Low Soil moisture on wheat root anatomy three weeks after germination. Roots were sampled 2cm from the tip (panels A and C) and 13cm from the tip (B and D). Panels display cortical cell file number at 2cm (A) and 13cm (B), cortical cell count at 2cm (C) and 13cm (D. Error bars represent ± SE and different letters represent significant differences at the 5% level after a post-hoc Fisher's unprotected least significant difference test. n=4

Wheat cortical cell file number (CCFN) measured at 2cm (Figure 13A) was significantly affected by soil moisture as a main effect ($P<0.001$), and an interaction that was close to significance at the 5% level ($P=0.05$). These results were further enhanced at 13cm (Figure 13B) whereby CCFN was significantly affected by both humidity and soil moisture($P=0.002$ and $P<0.001$ respectively) as well as a significant interaction between the two ($P<0.001$). Under low soil moisture conditions, high humidity led to significantly higher wheat CCFN.

Similar findings are presented with regards to the number of wheat cortical cells. At 2cm (Figure 13C), soil moisture had a significant main effect ($P=0.001$) as well as a significant interaction between humidity and soil moisture ($P=0.028$), as high humidity resulted in significantly more cortical cells during low soil moisture conditions. These findings were further supported by measurements 13cm (Figure 13D) from the root tip, whereby both humidity and soil moisture significantly affected the number of wheat cortical cells ($P=0.002$ and $P<0.001$ respectively) as a well as a significant interaction between the two ($P<0.001$). Again, high humidity resulted in significantly more wheat cortical cells when soil moisture was low.

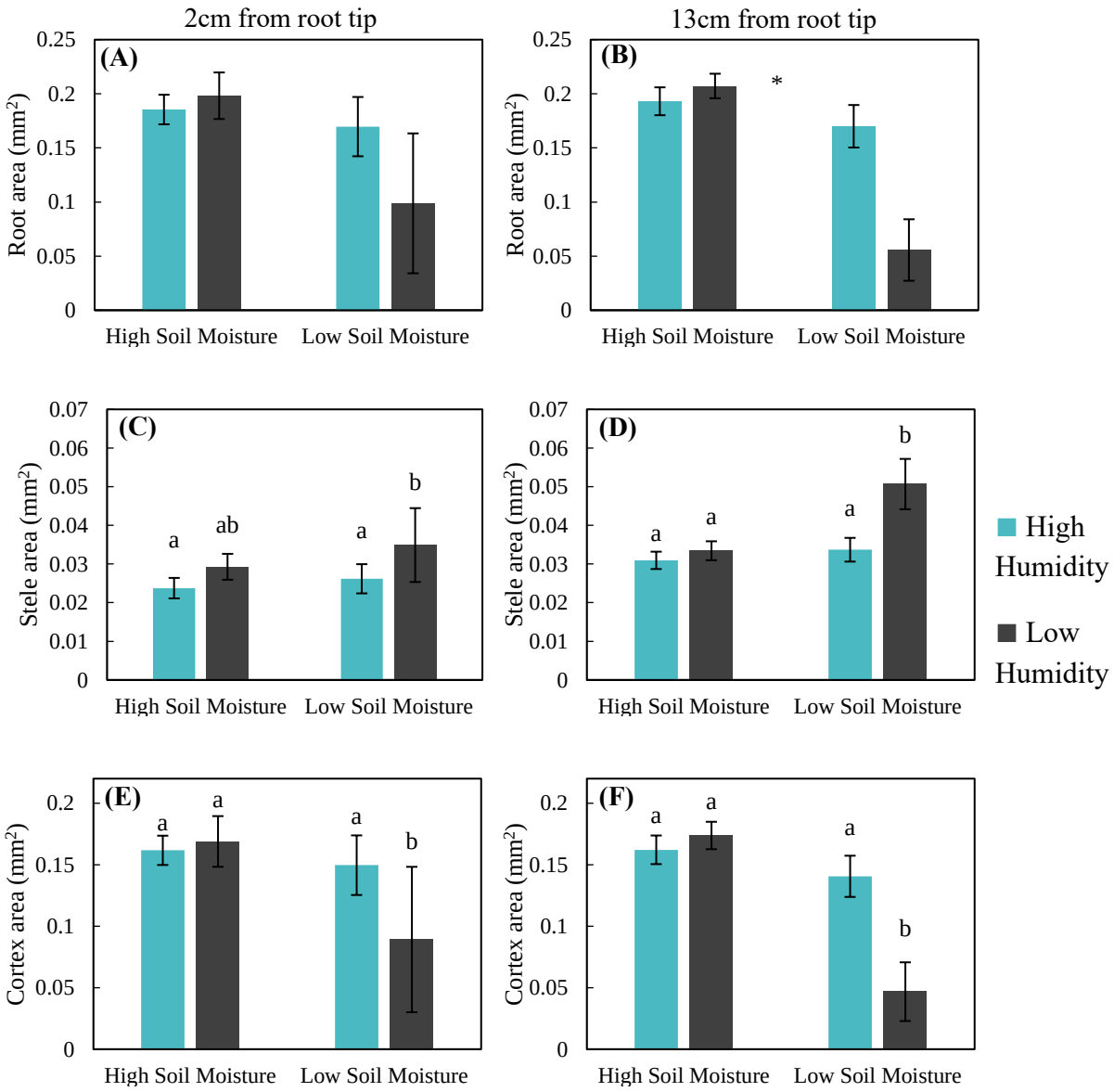


Figure 14. The effects of the four treatments High Humidity High Soil moisture, High Humidity Low Soil moisture, Low Humidity High Soil moisture and Low Humidity Low Soil moisture on wheat root anatomy three weeks after germination. Roots were sampled 2cm from the tip (panels A, C, E) and 13cm from the tip (B, D, F). Panels display whole root area at 2cm (A) and 13cm (B), stele area at 2cm (C) and 13cm (D), cortex area at 2cm (E) and 13cm (F), displayed as mm². Error bars represent ± SE and different letters represent significant differences at the 5% level after a post-hoc Fisher's unprotected least

significant difference test. * represents significant soil moisture main effect ($P<0.05$) after general ANOVA. n=4

Wheat whole root area measured 13cm away from the root tip (Figure 14B) was significantly smaller in low soil moisture conditions ($P=0.04$). Wheat stele area measured at 2cm (Figure 14C) was significantly lower in high humidity conditions ($P=0.22$). These results were further exaggerated at 13cm from the root tip (Figure 14D) whereby humidity and soil moisture significantly affected stele area ($P=0.011$ and $P=0.008$ respectively) as well as a significant interaction between humidity and soil moisture ($P=0.018$), whereby low humidity increased stele area in low soil moisture conditions. The area of the cortex at 2cm (Figure 14E) was significantly affected by soil moisture ($P=0.011$) and at 13cm these results were further enhanced whereby both humidity and soil moisture significantly affected cortex area ($P=0.005$ and $P<0.001$ respectively), as well as a significant interaction between the two ($P<0.001$) Under low soil moisture conditions, high humidity led to a significantly larger cortex area, reflecting cortical anatomy found in high soil moisture conditions.

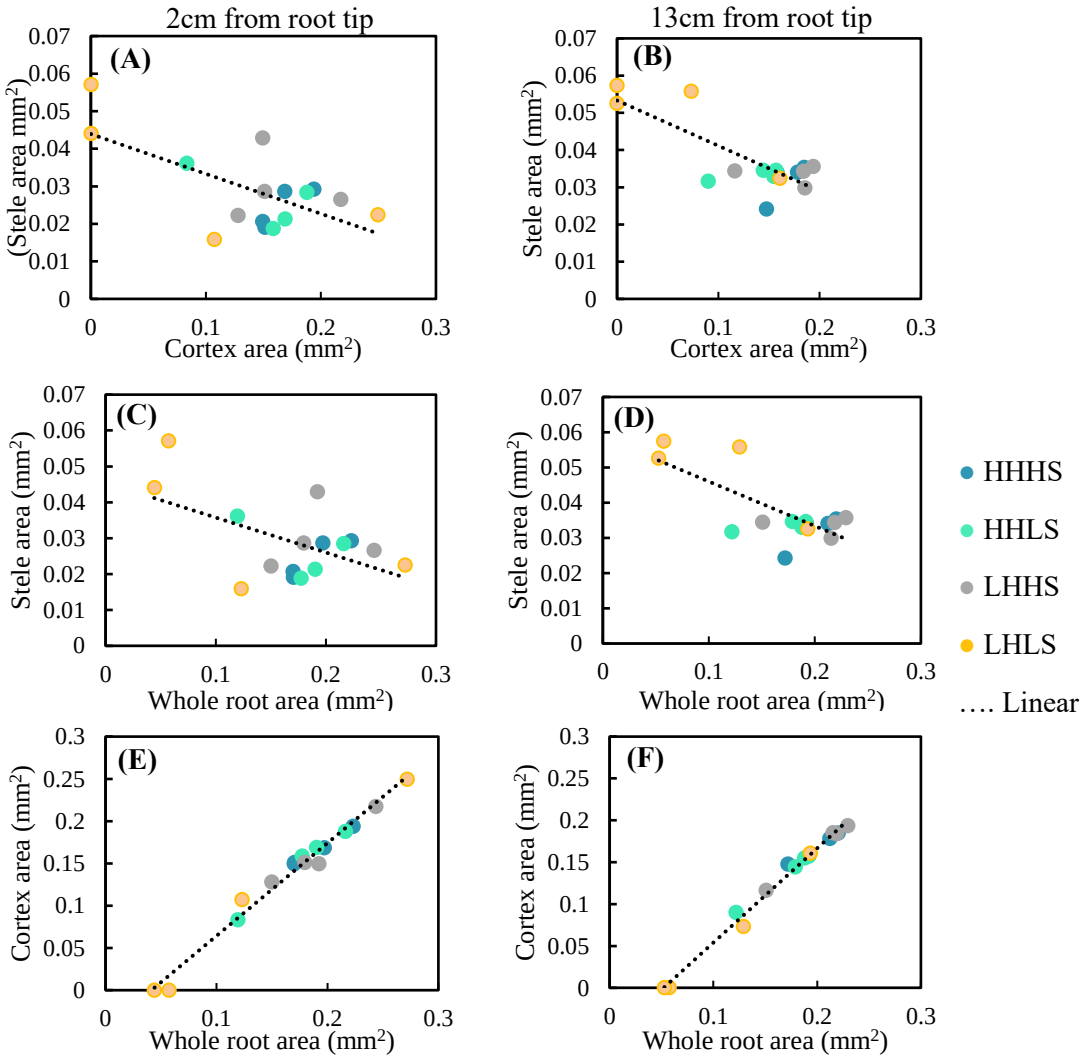


Figure 15. Scatter plots comparing wheat cortex area and stele area at 2cm (A) and 13cm (B) from the root tip, whole root area and stele area at 2cm (C) and 13cm (D), and whole root area and stele area at 2cm (E) and 13cm (F). Dotted line represents linear regression based on all data points, each treatment n=4, r^2 values (A) 0.419, (B) 0.6365, (C) 0.2867, (D) 0.5339, (E) 0.9808, (F) 0.9892.

There is a negative correlation between stele area and cortex area (Figure 15) when measured 13cm from the root tip (r^2=0.64). There is also a strong positive correlation between cortex area and whole root area when measured at both 2cm from the root tip (r^2=0.98) and 13cm from the root tip (r^2=0.99).

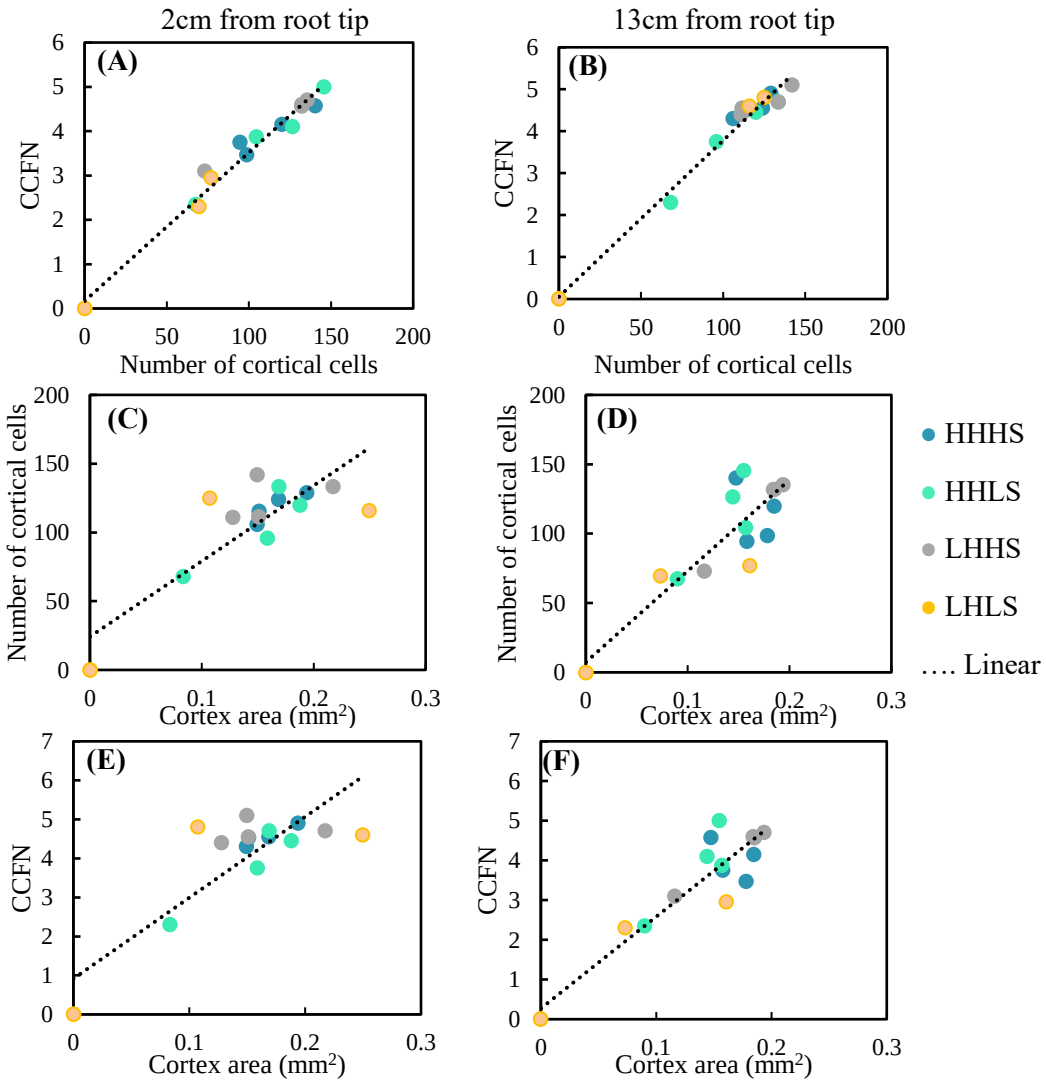


Figure 16. Scatter plots comparing wheat number of cortical cells and cortical cell file number (CCFN) at 2cm (A) and 13cm (B) from the root tip, cortex area and number of cortical cells at 2cm (C) and 13cm (D), and cortex area and CCFN at 2cm (E) and 13cm (F). Dotted line represents a linear regression model plotted, based on all data points (n=16), each treatment n=4, r^2 values (A) 0.9781, (B) 0.9816, (C) 0.74, (D) 0.8056, (E) 0.7454, (F) 0.8655.

There are strong correlations between wheat root anatomy in the cortex (Figure 16) measured at both 2cm and 13cm from the root tip r^2=(A) 0.9781, (B) 0.9816, (C) 0.74, (D) 0.8056, (E) 0.7454, (F) 0.8655.

4.4.2 Leaf Cuticle Chemistry

Results show that the ATR-FTIR spectroscopy technique was able to detect chemical compositional features of the leaf cuticle in both maize (Figure 5) and wheat. Displaying distinct peaks in wavebands associated with waxes, in particular those between 2800-2950, due to the asymmetric and symmetric stretching (Liu *et al.*, 2019) (Figure 5). However, the results of the PCAs carried out on maize and wheat showed very little variation explained by first few components, therefore a more powerful dimensionality reduction technique was carried out to explore the data in more detail, a t-SNE.

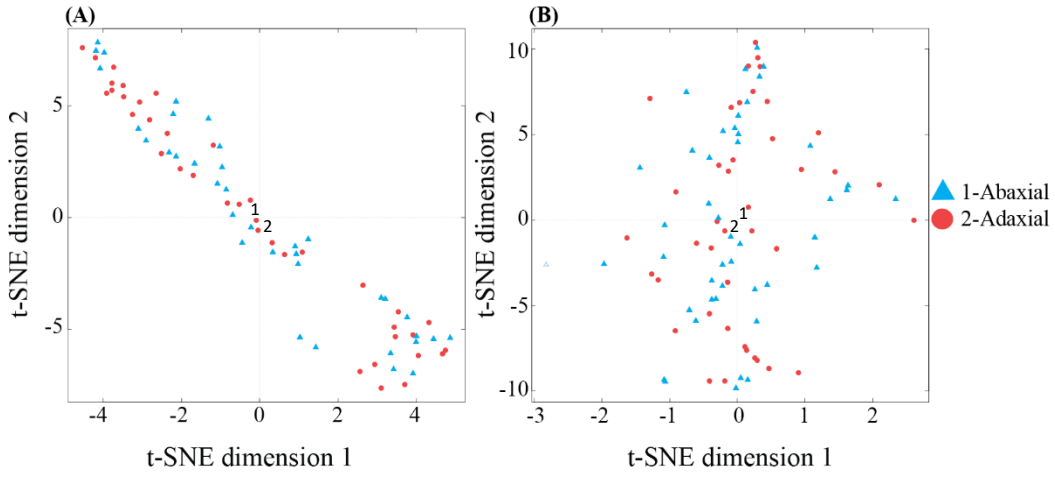


Figure 17. t-SNE investigating spectral differences between abaxial and adaxial sides of the leaf in maize (A) and wheat (B). Spectral analysis was carried out on plants three weeks after germination. t-SNE uses statistical embedding to reduce data features (the wavenumbers) to two-dimensional feature space. Numbers 1 and 2 dictate the centroids that represent the multi-dimensional average for abaxial and adaxial clusters respectively.

The t-SNE showed no distinct clustering between the abaxial and adaxial sides of the leaf in both maize and wheat (Figure 17), which suggested no distinct

differences in the compositional data between the abaxial and adaxial sides of the leaf, thus implying uniform cuticle chemistry across the whole leaf.

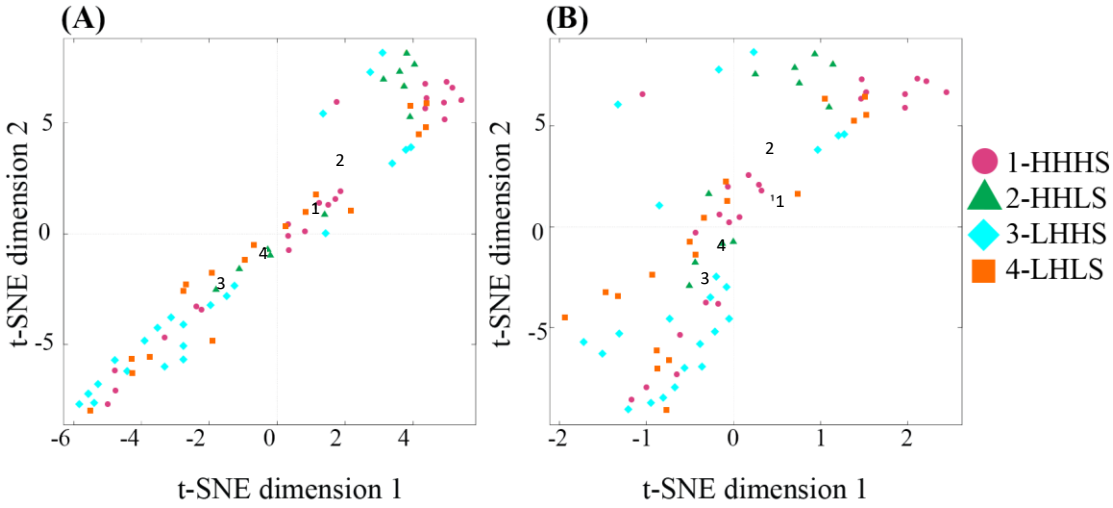


Figure 18. t-SNE two reproducible seeds (A) and (B) for maize showing both 1 and 2 centroids (HHHS and HHLS) are located in opposite quadrants than 3 and 4 (LHHS and LHLS), consistently across reproducible seeds. t-SNE uses statistical embedding to reduce data features (the wavenumbers) to two-dimensional feature space. Numbers 1,2,3 and 4 represent the centroids for HHHS, HHLS, LHHS, and LHLS clusters (or lack thereof) respectively, centroids represent the multi-dimensional average for the associated cluster.

The t-SNE carried out on maize (Figure 18) and wheat (data not shown due to complete lack of clustering and lack of possible centroid patterning shown in maize) showed no significant clustering around each of the four centroids (the multi-dimensional average for that cluster, represented by numbers 1-4) that indicated a lack of strong treatment effect on cuticle chemistry. If all four treatments had distinct effects on the chemistry data, we would have expected to see four distinct clusters around the centroids. Nevertheless, there could have been a very weak humidity effect in maize as, both 1 and 2 centroids (HHHS and HHLS) were located in opposite quadrants than 3 and 4 (LHHS and LHLS), such

a polar arrangement was consistent in various reproducible seeds of the t-SNE (Figure 18). These results suggested that the spectral absorbance of HHHS and HHLS grown plants was more similar to each other than to the low humidity grown (LHHS and LHLS) plants and vice versa. A possible 'divide' between high and low humidity grown plants irrespective of soil moisture (Figure 18). However, due to a lack of clustering, any differences caused by humidity were very weak.

Due to the potential but very weak treatment effects on maize, in particular humidity, and the associations between humidity effects on cuticle wax composition and deposition, a targeted Chi^2 analysis on peaks of interest (wavebands associated with wax composition and structure), was carried out. However, there were no significant treatment effects at the 5% level for both maize (Figure 19) nor were there any significant effects on wheat (Table 4).

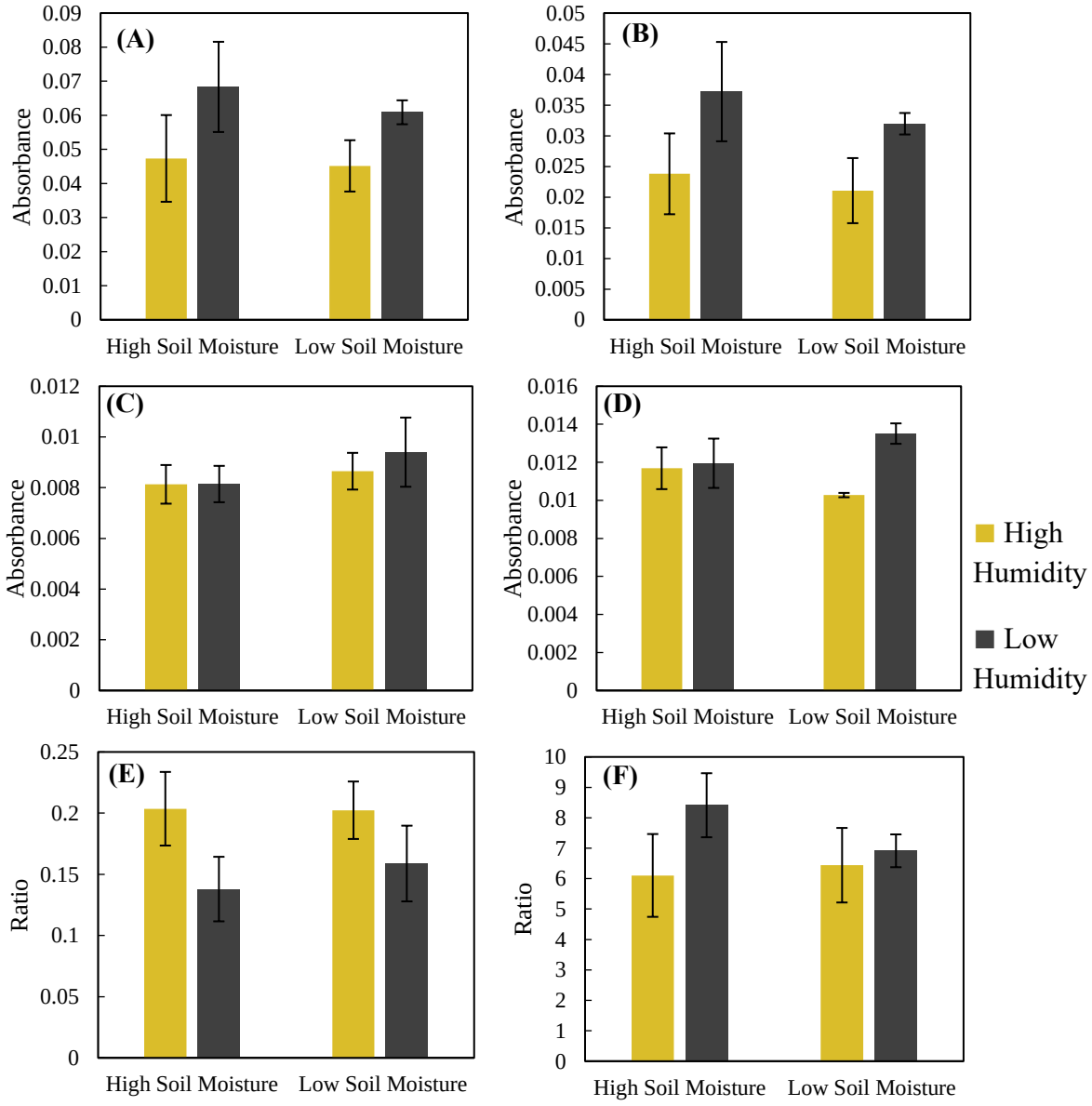


Figure 19. The effects of the four treatments High Humidity High Soil moisture (HHHS), High Humidity Low Soil moisture (HHLS), Low Humidity High Soil moisture (LHHS) and Low Humidity Low Soil moisture (LHLS) on the relative absorbance attained from ATR-FTIR Spectroscopy analysis on intact maize leaves three weeks after germination. The highest absorbance value between 5 wavebands around the peak of interest was compared for (A) 2925 ± 2 wavebands indicating CH_2 asymmetrical stretching, (B) 2840 ± 2 wavebands indicating CH_2 symmetrical stretching, (C) 1736 ± 2 wavebands indicating C=O, (D) 2954 ± 2 wavebands indicating CH_3, (E) ratio between C=O (C) and CH_2 asymmetrical stretching (A), and (F) displays the sum of CH_2 asymmetric and

symmetric stretching to CH_3 (A+B:D) ratio. Error bars represent SE, n= 4 for HHHS and LHHS and n=3 for HHLS and LHLS.

Table 4. Table displaying the Chi2 p-values from wheat spectral wavebands of interest. There was no significant relationship between the treatment conditions and any of the wavebands of interest at the 5% significance level.

Waveband	**Humidity Effect (Chi^2 p-value)**	**Soil Moisture Effect (Chi^2 p-value)**	**Humidity × Soil Moisture Interaction (Chi^2 p-value)**
2925 CH_2 asymmetric	0.651	0.541	0.236
2840 CH_2 symmetric	0.435	0.515	0.333
1736 C=O	0.686	0.623	0.814
2954 CH_3	0.748	0.868	0.179
C=O : CH_2 asymmetric ratio	0.617	0.617	0.245
Sum of CH_2 (asymmetric and symmetric):CH_3 ratio	0.7	0.559	0.827

4.5 DISCUSSION

4.5.1 Root Anatomy

Both maize and wheat responded differently in terms of anatomical adaptations to humidity and soil moisture, with some novel findings with regards to combined humidity and soil moisture effects also observed. In maize, whilst high humidity prevented the reduction in the number of metaxylem vessels when soil moisture was low, it, however, increased aerenchyma formation when soil moisture was

high. Whereas in wheat, high humidity prevented both enhanced root cortical senescence and an increase in stele area during low soil moisture conditions. Such changes to anatomy which are dependent on both soil moisture and humidity conditions highlight the importance of observing above and belowground environmental factors as influencers of root anatomy.

4.5.1.1 Maize

During low soil moisture and low humidity conditions, the decreased metaxylem number (Figure 6) could be a mechanism for reducing the capacity for water transport (Weerathaworn, Soldati and Stamp, 1992) in water-limiting, high transpirational demand conditions (driven by high VPD in low humidity conditions). Metaxylem vessels are efficient water conductors, large in diameter and allow a greater volume of water to pass through, an increase in the number of metaxylem vessels, therefore, increases root conductivity (Jaramillo-C, White and De La Cruz-A, 1992). As such, during water limiting conditions, previous studies have found the reduced metaxylem size in wheat (Richards and Passioura, 1989; Schoppach *et al.*, 2014), and the reduced number of metaxylem vessels in maize (Weerathaworn, Soldati and Stamp, 1992) are associated with increased resistance to water flow during water limiting conditions (Belford, Klepper and Rickman, 1987), and increased yields (Richards and Passioura, 1989). By lowering their hydraulic requirements during water limiting conditions, plants can increase water use efficiency and plant water potential, reduce hydraulic demand (Fichot *et al.*, 2009; Henry *et al.*, 2012; Kadam *et al.*, 2015) and subsequently reduce the risk of cavitation (Fichot *et al.*, 2009; Henry *et al.*, 2012; Kadam *et al.*, 2015). Therefore, the reduction in the number of maize metaxylem vessels in low soil moisture low humidity conditions is conducive to findings in the literature (Belford, Klepper

and Rickman, 1987; Richards and Passioura, 1989; Weerathaworn, Soldati and Stamp, 1992).

Interestingly, in this chapter, during low soil moisture conditions, high humidity maintained metaxylem numbers comparable to plants growing in high soil moisture conditions. The water-conserving reduction in metaxylem number strategy is not adopted in maize when humidity is high. It is possible that during this scenario (HHLS), the high humidity reduces the VPD experienced by the plant, therefore, reducing transpirational demand (Shamshiri *et al.*, 2018) and the pressure of root supplied soil water. The reduced demand for soil water leads to a growth strategy that is not focused on water conservation strategies, therefore a reduction in the number of metaxylem vessels is not needed.

Other alterations in maize root anatomy characteristics when both soil moisture and humidity were high, were reduced cortex area (Figure 8), subsequently reduced root area (Figure 8), and increased aerenchyma formation (Figure 7). As the number of cortical cells and cortical cell files directly influence the distance water and other soluble nutrients have to travel between the soil and the xylem, it is not uncommon in drought conditions for a plant to reduce the cortex area thus, reducing the distance travelled and metabolic costs of root growth thus improving drought tolerance (Chimungu, Brown and Lynch, 2014; Vadez, 2014). However, in this chapter, it is under ample water conditions with high humidity and high soil moisture (HHHS), that the reduced cortex and subsequent whole root area occurs (Figure 8). This was unexpected as it is low soil moisture that is considered to drive reductions in cortical cell area (Chimungu, Brown and Lynch, 2014;

Vadez, 2014), with low VPD (high humidity) found to increase whole root area in a tomato (*Solanum lycopersicum*) study (Zhang *et al.*, 2020).

The reduced cortex and whole root area in this chapter were possibly not driven by lack of water but rather lack of nutrient availability. High humidity (low VPD) can lead to a reduction in nutrient uptake via reduced transpiration (Leuschner, 2002; del Amor and Marcelis, 2005). A study on tomato (*Lycopersicon esculentum* Mill) (del Amor and Marcelis, 2005) found concentrations of phosphorous in the plant were reduced when grown at 95% relative humidity. If the high humidity conditions are leading to nutrient deficiencies such as phosphorous, this could explain maize's response to the HHHS conditions through reduced cortical area. Under low phosphorous conditions, root anatomy focused on reducing metabolic costs is favoured (Galindo-Castañeda, Brown and Lynch, 2018), and can improve plant growth in low phosphorous conditions. A study growing maize in low phosphorous conditions found reduced living cortical area improved soil exploration efforts, aided phosphorous capture and increased biomass. Therefore, it is possible that in this chapter, the low VPD in the HHHS treatment reduced transpiration and subsequent nutrient uptake, potentially resulting in nutrient deficiencies in immobile nutrients such as phosphorous. Maize responded by reducing living cortex area in a bid to reduce metabolic costs of effective soil exploration to improve nutrient uptake.

Reduced cortex area is not the only significant anatomical alteration to occur in the cortex. During high humidity conditions a significantly greater proportion of cortex area was occupied by aerenchyma when soil moisture was also high

(Figure 7), compared to virtually no aerenchyma present when soil moisture was low. These results are both comparable to previous studies and dissimilar to others but considering that aerenchyma play more than one role in the plant system, this agreement and contradiction to previous research is no surprise.

Aerenchyma can form under normal conditions, and during periods of abiotic stress (Mohammed *et al.*, 2019). During low soil moisture conditions, aerenchyma are beneficial for increasing plant drought tolerance (Zhu, Brown and Lynch, 2010), by maintaining root size but reducing the number of cells present thus reducing the metabolic costs of soil exploration (Zhu, Brown and Lynch, 2010). However, greater aerenchyma occupation in maize was not found in low soil moisture conditions (Figure 7), but high soil moisture, further exacerbated by high humidity. Though, as previously suggested the maize plants in HHHS could be experiencing nutrient deficiency from reduced transpiration and uptake from the soil, therefore the conversion of cortical cells to aerenchyma could have been employed to reduce the metabolic costs, by reducing the amount of living tissue, therefore maintaining more efficient root explorative growth to aid nutrient uptake e.g. phosphorous (Galindo-Castañeda, Brown and Lynch, 2018). However, if the aerenchyma formation in maize was driven by the need to reduce metabolic costs by maintaining root size and reducing the amount of living tissue (Zhu, Brown and Lynch, 2010), we would expect to see the lowest quantity and area of cortical cells when aerenchyma are present, as well as the larger roots (high whole root area). As such there are no such correlations when comparing aerenchyma area with cortical cell area and number of cortical cells (Figure 9) and the smallest whole root areas were found in the HHHS treatment plants (Figure 8). Therefore,

it is likely that the aerenchyma formation in maize could have been driven by something other than the need to reduce metabolic costs.

Aerenchyma are also widely considered to play a significant role in aiding gas movement around the plant (Mohammed *et al.*, 2019). Increased aerenchyma formation has been observed under hypoxic (He, Morgan and Drew, 1996) and waterlogged conditions (Armstrong, 2002; Colmer, Cox and Voesenek, 2006), as the air pockets help to supply oxygen from the shoots to the roots, during unfavourable conditions. This chapter shows high soil moisture results in a higher percentage occupation of aerenchyma in maize (Figure 7), further increased by high humidity. Perhaps, during these conditions whereby VPD is low and soil moisture content is high, the roots could be experiencing hypoxic conditions (though soils were not super saturated, which is usually indicative of hypoxic conditions), with shoots unable to supply enough oxygen to the roots due to reduced stomatal conductance caused by the low VPD (high humidity) conditions. The maize plants could be producing aerenchyma to aid oxygen supply rather than water uptake or metabolic cost reduction, similar to findings from a study on rice (*Oryza sativa*) (Henry *et al.*, 2012) and C4 tropical grasses (*Andropogon gayanus, Hyparrhenia rufa*, *Echninochloa polystachya*, and *Brachiaria mutica*) (Baruch and Merida, 1995), whereby aerenchyma formation decreased under drought.

The results of this study are therefore conducive to other findings, whereby low soil moisture led to a reduction in aerenchyma formation, leading us to believe that for maize, aerenchyma formation favours oxygen supply, rather than aiding drought tolerance in maize. Though if the HHHS treatment was exposing plants to

hypoxic conditions we would have expected to also observe aerenchyma formation in wheat in this treatment, as a study on *Triticum aestivum* (Huang *et al.*, 1994) observed aerenchyma formation during waterlogged conditions, this aerenchyma response was not present in wheat in this chapter. This does not disprove the presence of hypoxic conditions driving aerenchyma formation in maize, perhaps maize is more sensitive to these conditions. As Maize is more intolerant to hypoxia than wheat, though wheat is still moderately intolerant (Mustroph and Albrecht, 2003), and an overarching suggestive finding in this thesis is that maize is more sensitive to changes in humidity than wheat. Perhaps the severe intolerance to hypoxia and maize's heightened sensitivity to changes in humidity, compared to wheat, drove the increase aerenchyma formation in maize during HHHS conditions, but not in the less intolerant, less humidity sensitive wheat.

4.5.1.2 Wheat

With regards to changes in wheat root anatomy, both soil moisture and humidity significantly affected vascular tissues, root area and cortical cell death. Whilst high humidity appeared to increase metaxylem number in maize during low soil moisture conditions, such responses are species-specific, with wheat seeing humidity driven changes to other vascular tissue when soil moisture is high (Figure 12). During HHHS conditions the number of xylem phloem vessels in wheat increased, suggesting an increase in the capacity for water transport (Weerathaworn, Soldati and Stamp, 1992) during ample water conditions (Figure 12). However, during low soil moisture conditions humidity did not affect xylem number (unlike maize metaxylem response), implying the hydraulic demand imposed on the plant during dry soil conditions, are too great to be alleviated by

VPD alone, suggesting wheat with regards to changes in vascular tissue is more sensitive to soil moisture conditions than maize.

During low soil moisture and low humidity conditions (LHLS) wheat roots underwent root cortical senescence (RCS) (visually observed in Figure 3H, cortical cells quantified in Figure 13), whereby the outer layers of the root are completely lost, leaving the endodermis and the stele, thus reducing the whole root area. This was an expected result as cortical senescence, is a common phenomenon in cereals, particularly wheat during drought conditions (Lynch, Chimungu and Brown, 2014). In wheat, the cortex makes up a majority of the root area, with so many living cells the cortex is the most metabolically expensive part of root anatomy, achieving the term 'cortical burden' in studies assessing efficient soil exploration (Jaramillo *et al.*, 2013). This chapter also observed a negative relationship between cortex area and stele area (Figure 15), implying that more metabolically expensive roots (with larger cortex) also have the largest distances for soil-derived water and nutrients to travel from rhizosphere to xylem vessels. Cortical senescence, therefore, acts to reduce both the metabolic cost of soil growth and exploration but also the distances water and soluble nutrients need to travel to enter the xylem and the rest of the plant (Lynch, 2013, 2015; Lynch, Chimungu and Brown, 2014). As such, cortical senescence is a strategy adopted by wheat during severely water limiting conditions (Lynch, Chimungu and Brown, 2014). However, in this chapter, interestingly high humidity resulted in significantly more cortical cells in wheat during low soil moisture conditions, the roots appeared more comparable (in terms of cortex area) to wheat plants growing in high soil moisture conditions, cortical senescence was avoided in low soil

moisture conditions when humidity was high. Thus, suggesting that during low soil moisture conditions, high humidity (and subsequent low VPD) can reduce the transpirational demand of soil-derived water which would have previously led to radical drought tolerance adaptations such as cortical senescence.

During the low soil moisture conditions, low humidity (LHLS) resulted in a significantly larger stele area (Figure 14), with high humidity resulting in smaller stele areas that resembled plants growing in high soil moisture conditions. Increasing the proportional stele area over cortex under drought conditions could indicate that the plant is prioritising water retention in the vascular tissue, something that has previously been demonstrated in rice (Henry *et al.*, 2012). However, increased stele areas in drought studies are more commonly associated with improved root penetrability of hard soils with higher bulk densities (Chimungu, Loades and Lynch, 2015; Klein *et al.*, 2020), which could be indicative of the soil conditions that the wheat plants are experiencing in the low soil moisture treatments during this chapter. Though pots were packed with sandy soil to a bulk density of $1.3 gcm^{-1}$, the low soil moisture conditions could have increased soil hardest as soil strength increases non-linearly with decreasing soil moisture, thus increasing the penetrative resistance encountered by the roots. Therefore, the wheat could have adapted to the increased mechanical impedance of the soil by increasing the stele area and improving the penetrative ability of the roots. That being said, if this is the case, the high humidity conditions reducing stele area to sizes comparable to wheat growing in high soil moisture conditions (Figure 14), could be detrimental to the exploration of the drier, harder soils in the

low soil moisture treatment, with high humidity leading to the reduced penetrative ability of the root system.

Both humidity and soil moisture affected maize and wheat root anatomy, which could have significant impacts of nutrient acquisition, explorative metabolic costs, and water uptake/transport. It appears that high humidity (low VPD) could play an alleviating role to drought stress in both maize and wheat, by maintaining large numbers of metaxylem vessels in maize and preventing cortical senescence in wheat, when soil moisture is low. However, during high humidity conditions could be detrimental to dry soil exploration in wheat through reduced stele area and potentially reduced penetrative ability. Moreover, high humidity coupled with high soil moisture could result in hypoxic and/or nutrient-deficient conditions for maize, resulting in anatomical changes to roots which are characteristic of these conditions. Humidity and soil moisture treatment conditions, therefore, have the potential to significantly influence resistance and facilitation of water and nutrient uptake at the root level, as well as the acquisition of resources through effective soil exploration.

4.5.2 Cuticle Chemistry

With regards to cuticle chemistry there were no detectable differences in the compositional data between abaxial and adaxial leaf surfaces in maize and wheat, nor were any specific treatment effects detected. Though the maize absorbance data suggested a possible humidity effect (due to the centroid locations in opposite quadrants), though any effect would have been extremely weak, and no significant effects on the absorbance of wavebands were detected. There was a large amount of variation in the absorbance data, with larger error bars plotted (Figure 19),

perhaps a larger sample size, with plants grown in the conditions for longer whereby tissue from different developmental stages could be sampled, may have led to more significant findings.

At the leaf level, we see that an ATR-FTIR method is a useful tool for creating a spectral profile from fresh samples (Figure 5). However, during this chapter, humidity and soil moisture treatments had no significant effect on the spectral profile of the leaf cuticle of maize and wheat, nor did the treatments significantly affect any of the wavebands of interest. Though no other studies have looked at the effects of both humidity and soil moisture on leaf cuticle chemistry per se, experiments in previous studies on abiotic factors such as heat and illumination yielded significant differences to the leaf chemical spectra. Most recently, studies have detected significant chemical diversity in leaf cuticles spectra in field pea (*Pisum sativum* L.) (Liu *et al.*, 2019) and Arabidopsis (Liu *et al.*, 2020) grown under heat stress conditions compared to normal non-stressed conditions, and a study on kale (*Brassica oleracea*) observed a significant difference in cuticle chemistry when grown under high illumination (Shepherd *et al.*, 1997). Leaf chemistry can respond to abiotic factors, which can be reflected in changes to the chemical spectral profile of the individual, it was therefore somewhat expected that the treatment conditions albeit either soil moisture or humidity or an interaction between the two would yield some spectral differences in the plant's chemical spectral profile.

The lack of clustering in the PCA's and t-SNE's and lack of significance from the Chi^2 for both maize and wheat (Figure 19 and Table 4) could be due to the natural

variation in leaf chemistry of plants as well as age-related chemical differences, as cuticle chemistry varies between younger and older leaves (Ribeiro da Luz, 2006; Heredia-Guerrero *et al.*, 2014). A study on tomato (España *et al.*, 2014). observed considerable changes to the chemical spectra of the cuticle as the plant develops, whereby different stages of fruit development were characterised by spectroscopy, with the most spectral changes observed during ripening (España *et al.*, 2014). In this chapter, although the longest unfurled leaf was chosen to be sampled, these leaves could have varied slightly in age due to different growth rates (visual observation). Therefore, the relatively small sample size (n=3 and n=4) and the variation in chemistry between leaves of different ages, could have driven the relatively large variation and subsequent lack of significance, when investigating treatment effects. The lack of significance could also be due to the plants not being exposed to the treatment conditions for long enough, to invest in alterations to cuticle chemistry. A study on the effects of heat stress on field pea cuticle chemistry analysed samples when the plant had matured to flowering (Liu *et al.*, 2019), and a study on different water regimes on cuticle chemistry in Arabidopsis grew the plants in treatment conditions for four weeks (Liu *et al.*, 2020).

The very weak potential humidity effect for maize cuticle chemistry, with regards to the centroid locations from the t-SNEs of various reproducible seeds (Figure 18). Is not an unexpected result as humidity is known to influence leaf wax morphology, chemistry and the wettability of a leaf (Koch *et al.*, 2006). A study on *Brassica oleracea, Eucalyptus gunnii,* and *Tropaeolum majus* found that growth of plants in 98% relative humidity, all species exhibited a reduction in total wax mass and wax crystal density as well an increase in surface wettability

(Koch *et al.*, 2006). That being said, if humidity had a strong effect on cuticle chemistry in this chapter, we would have expected more significant clustering in the PCA's and t-SNE's caused by humidity and also differences in the relative absorbances of wavebands associated with wax content. Wavebands such as 2954cm^{-1} are indicative of CH_3, a recent study observed a significant positive correlation between absorbance and total wax accumulation (Liu *et al*., 2020). Similarly, the CH_2 asymmetric and symmetric stretching (2925 cm^{-1} and 2840 cm^{-1}) has a strong absorbance in waxes in the plant cuticle (Heredia-Guerrero *et al*., 2016). A study on Arabidopsis wax-less mutants and wild type relatives (Liu *et al*., 2020) recorded all five of the wax deficient mutants to exhibit smaller peaks in the CH_2 asymmetric and symmetric vibrations, further supporting the relationship between CH_2 bands (both asymmetric and symmetric) and cuticle wax content. Therefore, if humidity were to have a significant effect on cuticle wax content, we would have expected to have seen some significant differences in these wavebands, which was not the case.

4.6 CONCLUSION

This chapter highlights some of the anatomical changes employed by maize and wheat that was grown under high and low humidity regimes and how individual responses are also dependent on soil moisture content. It also highlights the lack of difference in cuticle chemistry between the abaxial and adaxial leaf surfaces as well as the lack of treatment effect on cuticular chemistry in both maize and wheat. Though the chemistry data for maize is a little more suggestive of potential humidity effects and requires further experimentation for a definitive conclusion.

Hypotheses revisited

1. Low soil moisture will promote root cortical aerenchyma (RCA) formation in maize.

We reject this hypothesis, for aerenchyma formation was greatest in maize during the high humidity high soil moisture (HHHS) treatment.

2. Low soil moisture will promote root cortical senescence (RCS) in wheat regardless of humidity.

We reject this hypothesis as root cortical senescence was reduced in low soil moisture conditions when humidity was high.

3. Low humidity (high VPD) will cause a reduction in stele area regardless of soil moisture content.
4. There will be no major differences between abaxial and adaxial cuticle chemistry within both maize and wheat.

We accept this hypothesis.

5. Humidity will cause alterations in cuticle chemistry which relate to wax deposition, regardless of soil moisture content.

We reject this hypothesis for wheat and maize despite hints at some changes to cuticle chemistry, no significant treatment effects were detected.

We reject this hypothesis for both maize and wheat. Whilst maize showed no significant difference in the stele area between treatments, wheat only witnessed reduced stele area in low soil moisture conditions when humidity was also low.

5 EFFECTS OF ATMOSPHERIC HUMIDITY AND SOIL MOISTURE ON ROOT SYSTEM ARCHITECTURE, GAS EXCHANGE AND BIOMASS PRODUCTION

5.1 INTRODUCTION

With a dramatically changing climate and an ever more increasing global population, expected to reach 10 billion by 2060 (Truong, McCormick and Mullet, 2017), global cereal crop productivity needs to increase by 39% to over 4 billion metric tons by 2050 (Shah and Wu, 2019). Investigating the effects of humidity and soil moisture on below and aboveground physiology in greater detail, will not only help us decouple the effects of humidity and soil moisture but also observe whether one can mitigate the effects of the other e.g. high humidity reducing the drought stress imposed on plants during dry soil moisture conditions. This chapter will therefore investigate belowground changes to root system architecture (RSA) and aboveground stomatal morphology and gas exchange, whilst also acknowledging above and belowground biomass production. Due to the more controlled growth conditions from the use of growth chambers in this experiment temperature and humidity are kept constant throughout the experiments more precisely than previous chapters, so that any changes to VPD can be attributed to changes in atmospheric humidity (water content), not temperature.

Root system architecture (RSA) refers to the spatial distribution, growth stage and characteristics of roots from a single plant (Zhu *et al.*, 2011). The main role of RSA is to optimise water and nutrient uptake (Nibau, Gibbs and Coates, 2008) through efficient foraging of the soil environment and by exhibiting a degree of

plasticity to environmental stimuli (Zhu *et al.*, 2011). Plant roots will generally grow towards areas of high soil moisture and away from high osmolarity to avoid salt stress (Takahashi *et al.*, 2003). RSA is sensitive to both abiotic and biotic stresses and is, therefore, an important physiological adaptation to investigate, when assessing plant responses to environmental conditions, and observing how conditions such as soil moisture content and humidity can affect resource acquisition from the rhizosphere and the subsequent effects on plant performance.

With few roots produced during embryogenesis and a majority emerging as the plant develops (Zhu *et al*., 2011), RSA is finely tuned with the surrounding environment. During water and/or nutrient limiting conditions plants tend to allocate a greater proportion of resources to root development (Fitter and Stickland, 1991) thus enhancing soil exploration in a variety of ways (Hepworth, *et al*., 2016). Accelerated lateral root formation and higher root hair densities, can increase the surface area of the RSA (Nibau, Gibbs and Coates, 2008; Hepworth, *et al*., 2016), thus increasing the extent of the soil-root interface (rhizosphere) and enhancing uptake of water and other resources, in particular, immobile nutrients such as phosphorus (Lambers, Atkin and Millenaar, 2002). In addition, soil exploration of varying depths can be affected by root type, as adventitious roots such as the crown roots found on maize are more capable of more efficient exploration of the upper soil layers (Nibau, Gibbs and Coates, 2008). However, prolific soil exploration is not without its costs in both resources and energy. Therefore, a plant growing in sub-optimal conditions must be frugal with reserves whilst finding the most efficient soil exploration strategy.

As resources can vary in their mobility in the soil environment (Barber, 1995), various RSA's can arise, in response to the prevailing resource conditions. Reduced lateral root formation and root diameters (Sharp *et al.*, 2004; Bauerle *et al.*, 2008; Hund, Ruta and Liedgens, 2009; Lynch, 2013) can ease metabolic costs during low water and/or nutrient environments. Deeper root penetration can help unlock previously inaccessible soil water reserves (Bauerle *et al.*, 2008; Nibau, Gibbs and Coates, 2008; Henry *et al.*, 2012), is considered a key strategy for desiccation avoidance (Hund, Ruta and Liedgens, 2009). This is especially pertinent in drought conditions whereby, upper soil dry out first, with deeper soil layers retaining valuable moisture (Bauerle *et al.*, 2008). Whilst shallower foraging favours root systems growing in phosphorous limiting environments as phosphorus is relatively immobile and commonly confirmed to the upper layers of the soil strata. In cases of extreme resource deficiency, significantly hindered root growth can occur, ensuing shallower, less dense root systems with reduced total root lengths (Rich and Watt, 2013).

The knowledge gained from investigating the responses of RSA to changes in environmental conditions such as humidity and soil moisture could aid the development of new crop cultivars with enhanced root foraging capacity and resource acquisition in sub-optimal conditions. A strategic goal, as we face resource depletion, climate change, and a booming global population. However, The RSA is only one side of the story, to understand the effects of humidity and soil moisture on whole plant physiology, we need to set our sights a little higher and investigate how such conditions can affect overall water uptake, gas exchange and subsequent productivity. Since water uptake is a function of not only the RSA

but also soil and root hydraulic conductance and transpirational demand (Henry *et al.*, 2011) which in turn is also affected by VPD. There must be a form of communication between the roots and shoots and vice versa (Nibau, Gibbs and Coates, 2008), meaning that above and belowground stresses are not only 'felt' and confined to their source environments, but can affect whole plant physiology.

Evidence of aboveground conditions impacting belowground organs comes from a study on *Senecio vulgaris* which found that at elevated atmospheric CO_2 concentrations (700 μmol mol^{-1}) roots were longer, more heavily branched and foraged through a larger volume of soil (Henry *et al.*, 2011). Possibly as a result of the higher carbon supply reducing the costs of tissue production and plant growth. The study also claimed that above-ground conditions (high CO_2 concentrations) could help to mitigate the effects of belowground stresses (low soil moisture) as root systems grown under elevated CO_2 and low water supply had similar branching and foraging patterns as those grown under ambient CO_2 with high water supply. Likewise, changes to above-ground processes can also influence belowground root systems. Hepworth *et al.*, (2016) found that plants with higher stomatal densities and stomatal conductance exhibited a larger rooting area, resulting in greater phosphate uptake capacity, whereas low stomatal conductance resulted in small root systems. Therefore, if we are to understand the impacts of atmospheric humidity and soil moisture on the whole plant system, we need to also investigate the responses of aerial organs in terms of gas exchange and photosynthetic capacity, whilst exploring potential relationships between above and belowground processes.

This chapter will also explore how soil moisture and humidity affect plant water dynamics and productivity in terms of stomatal conductance, photosynthetic capacity, water use efficiency, and carbon assimilation. Changes to photosynthetic parameters and leaf morphology can affect whole-plant productivity, with reductions in photosynthetic ability associated with decreased yield potential (Zheng *et al.*, 2019). Higher NPQ (non-photochemical quenching) can be a sign that plants are experiencing inhibited photosynthetic processes due to abiotic and biotic stress, whilst leaf temperatures can affect critical photosynthetic enzymes (e.g Rubisco) (Salvucci and Crafts-Brandner, 2004).

Through the investigation of aboveground, leaf-level and canopy-level traits such as canopy area, we can begin to search for potential relationships between aerial and subterranean organ development in response to treatment conditions. Furthering our understanding of whole-plant physiological responses to humidity and soil moisture, with regards to resource acquisition (e.g. radiation and water), and resource application (plant growth and development) as well as water use efficiency. Water use efficiency is a critical crop parameter when developing crop simulation models, as it is the relationship between crop carbon gain and water usage (Steduto and Albrizio, 2005). Due to current climatic conditions, water shortage in crop systems is an escalating issue. Understanding which factors play a role in a plants water use strategy and how the plants are influenced by perturbations in climatic conditions such as changes to soil moisture availability or relative humidity could go a long way in informing crop breeding and plant management practices. Some research suggests that limited supplemental irrigation during the growing season can increase water use efficiency in wheat

(Waraich and Ahmad, 2010), thus highlighting the importance of understanding how irrigation practices and growth conditions (e.g. relative humidity) could influence water use efficiency and ultimately crop yield.

Building upon findings from Chapter 2, this current chapter will investigate root system architecture, photosynthesis, and gas exchange responses to treatment conditions in greater detail, by harnessing the technology of μCT and Licor, and by cultivating plants in controlled growth chambers.

5.2 AIMS AND HYPOTHESES

This chapter will build upon experimental findings from Chapter 2 in further detail whilst investigating the potential for high humidity to 'mitigate' the effects of low soil moisture on plant physiology, in both maize and wheat. Therefore, this chapter will test the following hypotheses.

1. Root system architecture will be unaffected by humidity

2. Low soil moisture will result in increased rooting depths.

3. Maize stomata will remain open in high humidity regardless of soil moisture content.

4. Wheat stomata will remain close under low soil moisture, regardless of humidity.

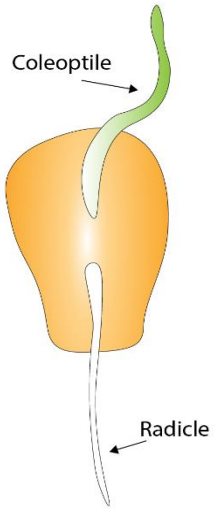
5. High humidity will increase ΦPSII in maize

6. Intrinsic water use efficiency (WUE) will be highest in plants grown under low soil moisture conditions.

5.3 MATERIAL AND METHODS

5.3.1 Plant Material and Experimental Design

40 maize (*Zea mays*) and 40 wheat (*Triticum aestivum* cv. Paragon) seeds were pre-germinated on blue roll wrapped in cling film in the high humidity chamber (Conviron A2000, growth conditions detailed below). 40 polypropylene columns (height 25.5cm, radius 4cm) (Chapter 2 Figure 1) were packed with sandy loam collected from a field site in Bunny, Leicestershire (Longitude 1.12608866, Latitude 52.86098725) and mixed with sand to a ratio of 50:50, to aid CT image acquisition.



The columns were packed to a bulk density of 1.3 g cm^{-3}. Five days after germination (upon the emergence of coleoptile and radicle), 20 maize and 20 wheat seeds were sown into the columns and randomly arranged in the treatment conditions (Table 1). Five reps of maize and wheat for all four treatments were grown for three weeks in controlled growth chamber conditions (Conviron A2000), with 12-hour day/night cycles, as per temperature and humidity conditions detailed below (Figure 1). Soil moisture treatment conditions (field capacity) were maintained with regular watering and weighing. The temperature was controlled to a greater degree than previous experimental chapters which were carried out in the glasshouse growth chamber. Air vapour pressure deficit was calculated using the following two equations from (Jones, 1992), as detailed in Chapter 2.

Due to leaf temperature measurements been taken at approximately 13:00 on the day of measurement, leaf VPD can be calculated for a given time for each of the

treatments and species. Leaf vapour pressure deficit was calculated by the following equation

$$Leaf\ VPD = Leaf\ SVP - \left(Air\ SVP \times \frac{RH}{100}\right) \qquad Equation\ 3$$

Where leaf SVP is calculated the same as Air SVP following Equation 1 using leaf temperature, not air temperature.

Maize and wheat were chosen as C4 and C3 representatives from the Gramineae family. Both are cereals but differ functionally in terms of carbon fixation mechanism and location on the isohydricity spectrum

Table 1. Treatment growth conditions. Relative humidity and temperature measurements were recorded using a Fisher Scientific Traceable Humidity/Temperature. Dew-Point Meter (Fisher, UK). Day refers to 06:00 – 18:00 and night (18:01 – 05:59). Soil moisture treatment was maintained with regular watering to weight. Air and leaf VPD calculated using equations 1,2 and 3. Leaf VPD represents a single point measurement at ~13:00, as leaf temperature measurements were only taken at one time point.

Treatment	Relative Humidity (%) (day/night)	Soil moisture (field capacity %)	Temperature °C (day/night)	Calculated Air Vapour Pressure Deficit (VPD_{air} kPa) (day/night)	Calculated Leaf Vapour Pressure Deficit (VPD_{leaf} kPa) at 13:00	
					Maize	**Wheat**
High Humidity High Soil Moisture **(HHHS)**	76.01/90.86	70	23.46/15.63	0.7/0.18	0.58	0.27
High Humidity Low Soil Moisture **(HHLS)**	76.01/90.86	30	23.46/15.63	0.7/0.18	0.46	0.65
Low Humidity High Soil Moisture **(LHHS)**	40.49/46.53	70	23.06/15.62	1.67/0.95	1.86	1.60
Low Humidity Low Soil Moisture **(LHLS)**	40.49/46.53	30	23.06/15.62	1.67/0.95	1.50	1.64

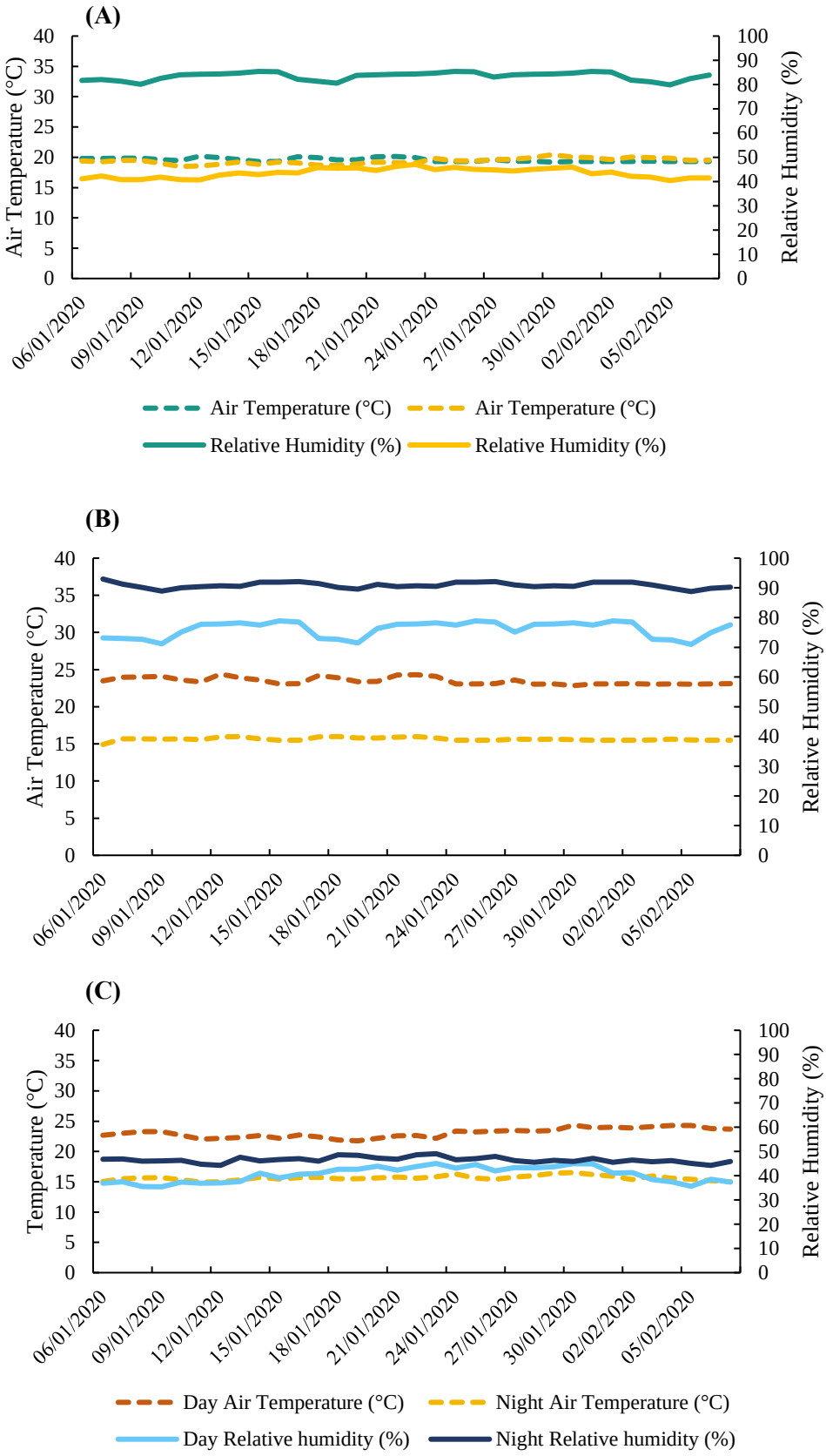


Figure 1. (A) Daily averages of the growth conditions in the high humidity and low humidity growth chamber measured using a Fisher Scientific Traceable

Humidity/Temperature. Dew-Point Meter (Fisher, UK). (B) Day and night averages for high humidity chamber and (C) low humidity chamber. Measurements made throughout the course of the experiment. Day refers to 06:00-18:00 and night to 18:01-05:59.

5.3.2 Root Architecture Visualisation in Situ Using µCT

For µCT analysis of root system architecture (RSA), three reps from each treatment were placed in a Phoenix Nanotom micro X-ray CT scanner (GE Sensing and Inspection Technologies, GmbH, Wunstorf, Germany) based at the Hounsfield Facility, University of Nottingham. This technique was chosen due to the non-destructive and accurate quantification of root systems that such scans can provide (Tracy *et al.*, 2012). Two scans were obtained from each sample (both a top half and a bottom half scan of the columns, which were later digitally stitched together to help maximise the resolution) with 1940 projection images (image averaging 3 and skip 1), using detector timing of 250 ms and X-ray setting of 174 kV and 200µA and a 0.2mm copper filter. The distance of the sample (FOD) and detector (FDD) from the source were 266.076 mm and 818.698 mm respectively, resulting in a spatial resolution of 0.064mm. Total scan time was 90 minutes

5.3.2.1 Image Reconstruction

Volume reconstruction and stitching of top and bottom scans were carried out in VG Studio MAX v.2.2.5 (Volume Graphics GmBh, Heidelberg, Germany) with a beam hardening correction set at eight. Individual adjustments were made to account for minor sample displacement and assure seamless stitching of images, as two volume sections are reconstructed to produce one volume for each sample.

5.3.2.2 Image Segmentation

Whole three-dimensional visualisations of maize and wheat root systems grown within the four treatments, were analysed based on rendered X-ray CT data in VG

Studio MAX v.2.2.5 (Volume Graphics GmBh, Heidelberg, Germany). Due to significant time constraints of fully rendering each sample, each scan, had a dedicated six hours of segmentation, to identify roots. After the allotted time, the sample was deemed 'complete' and analysis commenced on the next sample.

5.3.2.3 Root Architecture Analyses

Root volume and root surface areas were measured within VG Studio MAX v.2.2.5 (Volume Graphics GmBh, Heidelberg, Germany), and rooting depth was taken from the measured z-dimension within VG Studio Max v.2.2.5 (Volume Graphics GmBh, Heidelberg, Germany). Total root lengths were measured in RooTH 0.5.94 beta 3 software, from exported root volumes from VG Studio MAX v.2.2.5 (Volume Graphics GmBh, Heidelberg, Germany).

5.3.3 Stomatal Impressions and Analyses

One large stomatal peel was taken from each side of the leaf on five reps (in some cases 4 reps were available due to plant death) for each treatment and where possible, main veins avoided. Clear nail varnish was applied and left to dry, then gently removed with Sellotape and placed on a microscope slide.

Stomatal counts were made directly down the scope field of view at ×16 magnification for maize and ×10 magnification for wheat. Four fields of view were counted for each side of the leaf, they were then averaged to give one abaxial and one adaxial biological rep per plant. Maize n=5 for all treatments except LHHS where n=4. Wheat n=5 for HHLS and LHLS, and n=4 for HHHS and LHHS.

Stomatal dimension measurements were made on images acquired using IS Visicam Analyser software, on a ×25 magnification of the light microscope (as described in Chapter 2). Six stomata per side of leaf were measured then averaged for all the leaves in that treatment. Stomatal size (defined here as guard cell length multiplied by the total width of the guard cell pair as described in (Franks and Beerling, 2009) were measured in open source software Fiji (Schindelin *et al.*, 2012) with the scale set from a graticule image as discussed in Chapter 2. During the stomatal measurements, stomatal aperture was recorded as either open or closed (as described in Chapter 2 methodology 2.3). Stomata reported as open were done so regardless of the degree of stomatal opening. Stomata were reported closed were completely shut, encompassed by two turgid guard cells (see Figure 5 Chapter 2). Maximum stomatal conductance (g_{smax}) was calculated using an equation from (Franks and Beerling, 2009), detailed in Chapter 2 methodology (2.3). As the g_{smax} of water and CO_2 is proportional, only the g_{smax} of water values will be presented in the results.

5.3.4 Leaf Temperature

Before plants underwent stomatal conductance measurements, leaf temperature was recorded using an infrared thermal gun (IR KM823). A total of three spot measurements per plant were taken, close to the leaf surface, that were later averaged.

5.3.5 Leaf Conductance, Photosynthesis, and Chlorophyll Fluorescence (Spot Measurement)

Leaf conductance, photosynthesis, and chlorophyll fluorescence parameters were measured on the largest fully expanded leaf using the LI-6800 portable photosynthesis system (LI-COR, Nebraska, USA). Two 5-min spot measurements

were made on each plant, one at light level 500 μmol m^{-2} s^{-1} and the other at 1500 μmol m^{-2} s^{-}.

These low and high light levels were chosen to see how the plants responded to each level and to help decide on an appropriate light level for future experiments. Though 1500 μmol m^{-2} s^{-} would most likely be saturating, I wanted to see how the plants also performed under a lower, 500 μmol m^{-2} s^{-1} light level. Environmental conditions inside the cuvette throughout the measurement period were 23°C (air temperature), 400ppm CO_2, relative humidity 55%, and reference flow 500 μmol s^{-1}.

When all stomatal impressions, gas exchange and chlorophyll fluorescence measurements, and CT scans were complete, plant material was harvested, and biomass measurements were made.

5.3.6 Biomass and Area Measurements

Both root and shoot fresh weights were recorded separately. Dry weights were obtained through oven drying the plant material for four days at 70°C. A leaf section (~2cm in length) was excised from each plant and weighed. The leaf section was then placed under a Perspex sheet, flattened, and photographed. Then, the sample was then submerged in de-ionised water, in darkness at 5°C overnight, to produce standard turgor. The following day, samples were surface pat-dried using tissue and weighed immediately. Samples were then oven-dried for four days at 70°C, then weighed again.

The following calculations were carried out:

$$Specific\ leaf\ area\ (SLA) = \frac{Area\ of\ leaf\ (mm^2)}{Dry\ mass\ of\ leaf\ (mg)} \qquad Equation\ 5$$

$$Relative\ water\ content\ (\%) = \frac{(FW - DW)}{(TW - DW)} \times 100 \qquad Equation\ 6$$

$$Specific\ leaf\ mass = \frac{1}{SLA} \qquad Equation\ 7$$

$$Leaf\ weight\ ratio = \frac{Fresh\ weight\ (mg)}{Leaf\ area\ (mm^2)} \qquad Equation\ 8$$

$$Percentage\ leaf\ dry\ weight = \frac{Dry\ weight\ (mg)}{Fresh\ weight\ (mg)} \times 100 \qquad Equation\ 9$$

Where FW = fresh weight of samples when first collected, TW = turgid weight of samples after saturated with water, DW = dry weight of the sample after oven drying.

5.3.7 Statistical Analyses

All statistical analyses were carried out in Genstat 20th Edition. Treatments and effects were compared using a general ANOVA with main effects of soil moisture and humidity tested as well as any significant interaction between the two at the 5% level. When significance was detected, post hoc Tukey tests were carried out, when data was unbalanced Fisher's unprotected least significant difference test was used. For the stomatal aperture data (open/closed), a Chi-squared test was carried out to test for a relationship between the proportion of open/closed stomata and the treatment conditions. For whole-plant physiology correlations, a Pearson's correlation matrix was produced, with two-tailed significance presented at both the 0.05 and 0.001 level.

5.4 RESULTS

5.4.1 Root System Architecture

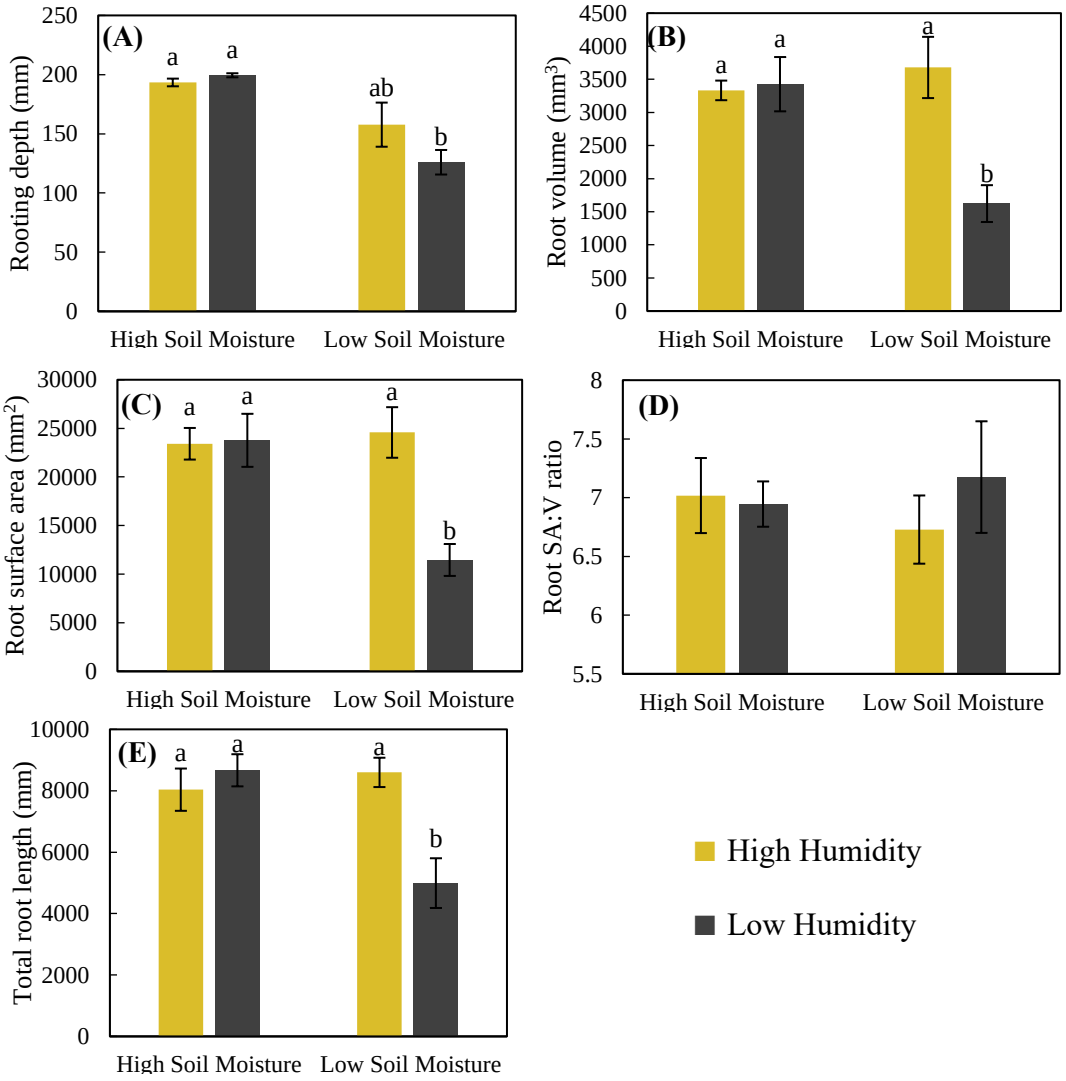


Figure 2. The effects of the four treatments High Humidity High Soil moisture, High Humidity Low Soil moisture, Low Humidity High Soil moisture and Low Humidity Low Soil moisture on maize root architecture traits measured during µCT analyses of maize plants three weeks after germination. (A) Rooting depth (mm), (B) Root volume (mm^3), (C) Root surface area (mm^2), (D) Root surface area (SA) to volume (V) ratio (SA: V), (E) Total root length (mm). Means are plotted, error bars represent ±SE. Different letters represent significant difference after post-hoc Tukey test. n=3.

In maize, low soil moisture led to significantly shallower root system architectures (Figure 2A) ($P<0.001$), compared to maize in high soil moisture conditions. Maize root volume (Figure 2B) surface area (Figure 2C) and total root length (Figure 2E) were significantly affected by humidity as a main effect ($P=0.022$, $P=0.02$ and $P=0.048$ respectively) and a significant interaction between soil moisture and humidity was noted ($P=0.015$ and $P=0.016$, $P=0.011$ respectively). During low soil moisture conditions, high humidity resulted in increased root volume (Figure 2B), root surface area (Figure 2C) and total root length (Figure 2E), compared to the low humidity low soil moisture grown plants.

Visual interpretation of maize root architectures (Figure 3), supported measured findings (Figure 2). Root systems from maize plants grown under low soil moisture conditions (Figure 3 B and D) showed shallower growth compared to those in high soil moisture conditions (Figure 3 A and C). Furthermore, under low soil moisture conditions (Figure 3 B and D) there was a visual difference between those above the blue dashed line (high humidity) and those below (low humidity), with observations of greater root growth seen in the high humidity (Figure 3 B) compared to low humidity conditions (Figure 3 D).

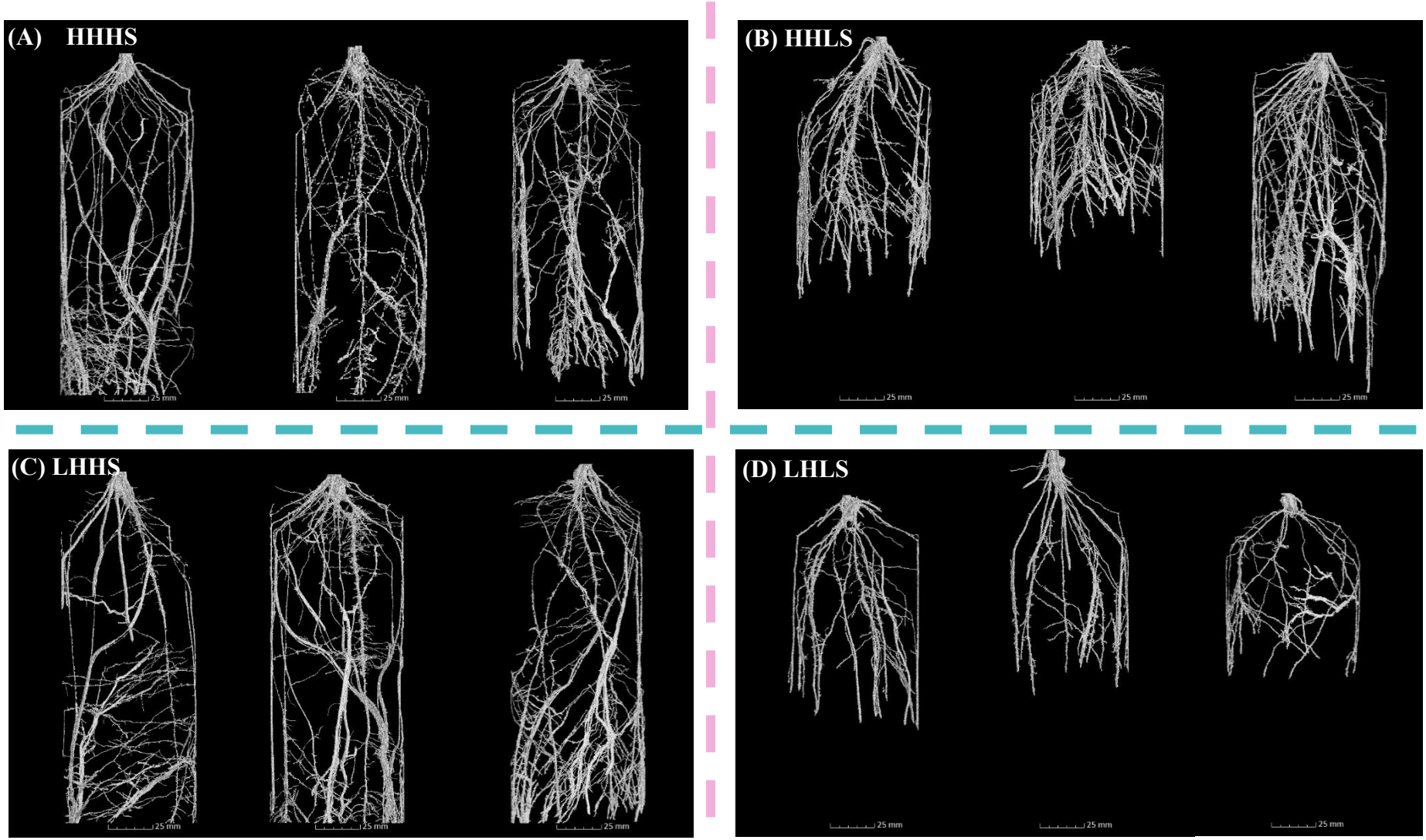


Figure 3. Rendered 3D maize root architectures produced in VG Studio MAX v.2.2.5 (Volume Graphics GmBh, Heidelberg, Germany). Each sample displays the detected root architecture from six hours of rendering. Maize root samples, three weeks after germination, and growth in the following treatment conditions: (A) High Humidity High Soil Moisture (HHHS), (B) High Humidity Low Soil Moisture (HHLS), (C) Low Humidity High Soil Moisture (LHHS), and (D) Low Scale bars represent 25mm.

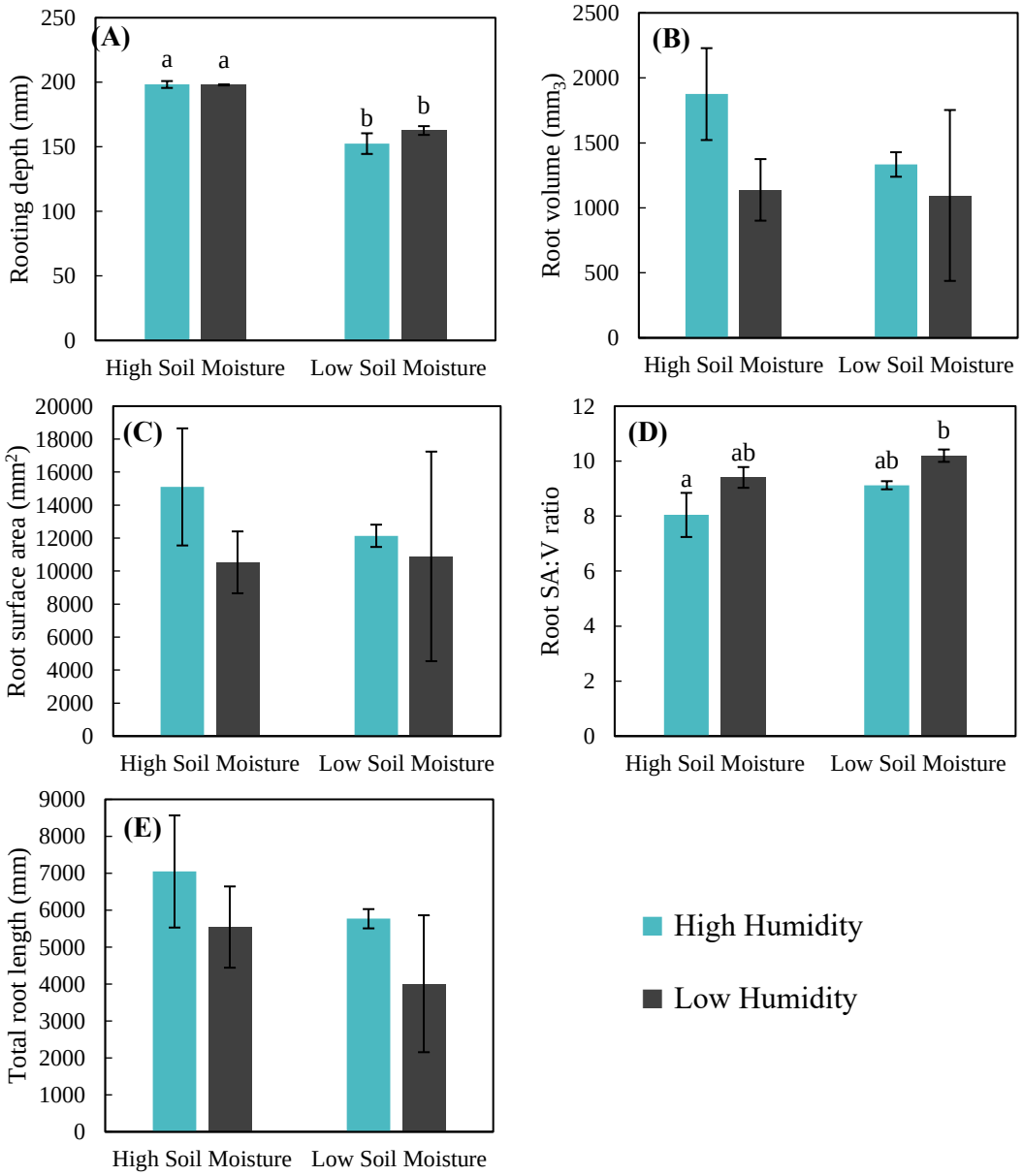


Figure 4. The effects of the four treatments High Humidity High Soil moisture, High Humidity Low Soil moisture, Low Humidity High Soil moisture and Low Humidity Low Soil moisture on wheat root architecture traits measured during µCT analyses of wheat three weeks after germination. (A) wheat rooting depth (mm), (B) wheat root volume (mm^3), (C) wheat root surface area (mm^2), (D) wheat root surface area (SA) to volume (V) ratio (SA: V), and (E) wheat total root length (mm). Means

are plotted, error bars represent ±SE. Different letters represent significant difference after post-hoc Tukey test between treatments. n=3.

Similar to the findings in maize, wheat roots were significantly shallower (Figure 4A) when grown in low soil moisture conditions ($P<0.001$), unlike maize, root surface area, volume, and total root length were not affected by treatment conditions. However, the wheat surface area to volume ratio (SA:V) (Figure 4D) was significantly affected by humidity ($P=0.03$) with high humidity resulting in significantly lower SA:V.

Comparable to maize, in the visual representation of the RSA in wheat (Figure 5) samples grown under low soil moisture conditions (Figure 5 B and D), showed shallower RSA's compared to those grown under high soil moisture conditions (Figure 5 A and C). There appeared to be a greater amount of variation within treatments in the number of roots detected and subsequently displayed when compared to maize, this could have driven the lack of significance measured in wheat root architecture traits.

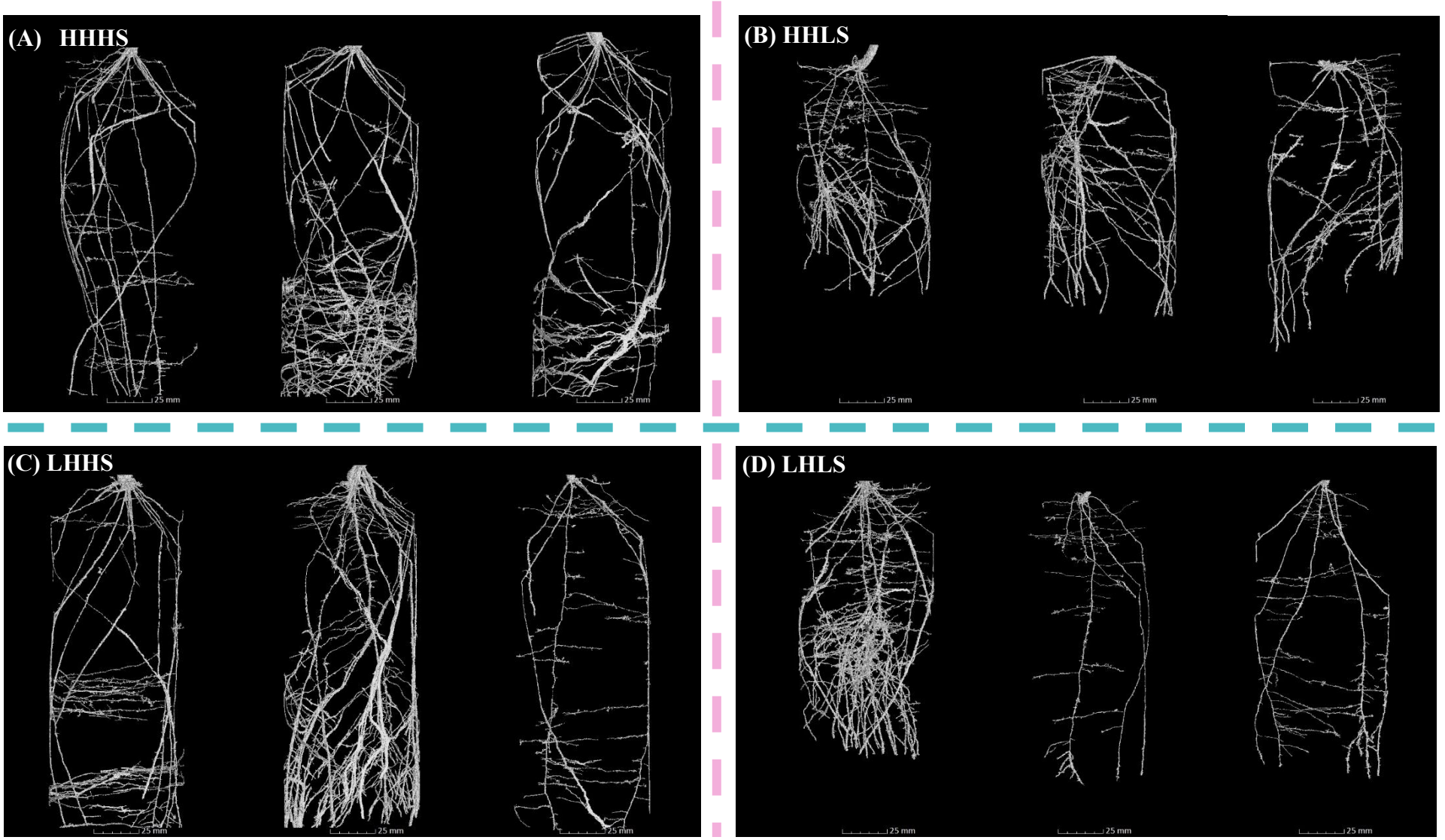


Figure 5. Rendered 3D wheat root architectures produced in VG Studio MAX v.2.2.5 (Volume Graphics GmBh, Heidelberg, Germany). Each sample displays the detected root architecture from six hours of rendering. Wheat root samples, three weeks after germination, grown in the following treatment conditions: (A) High Humidity High Soil Moisture (HHHS), (B) High Humidity Low Soil Moisture (HHLS), (C) Low Humidity High Soil Moisture (LHHS), and (D) Low Scale bars represent 25mm.

5.4.2 Gas Exchange

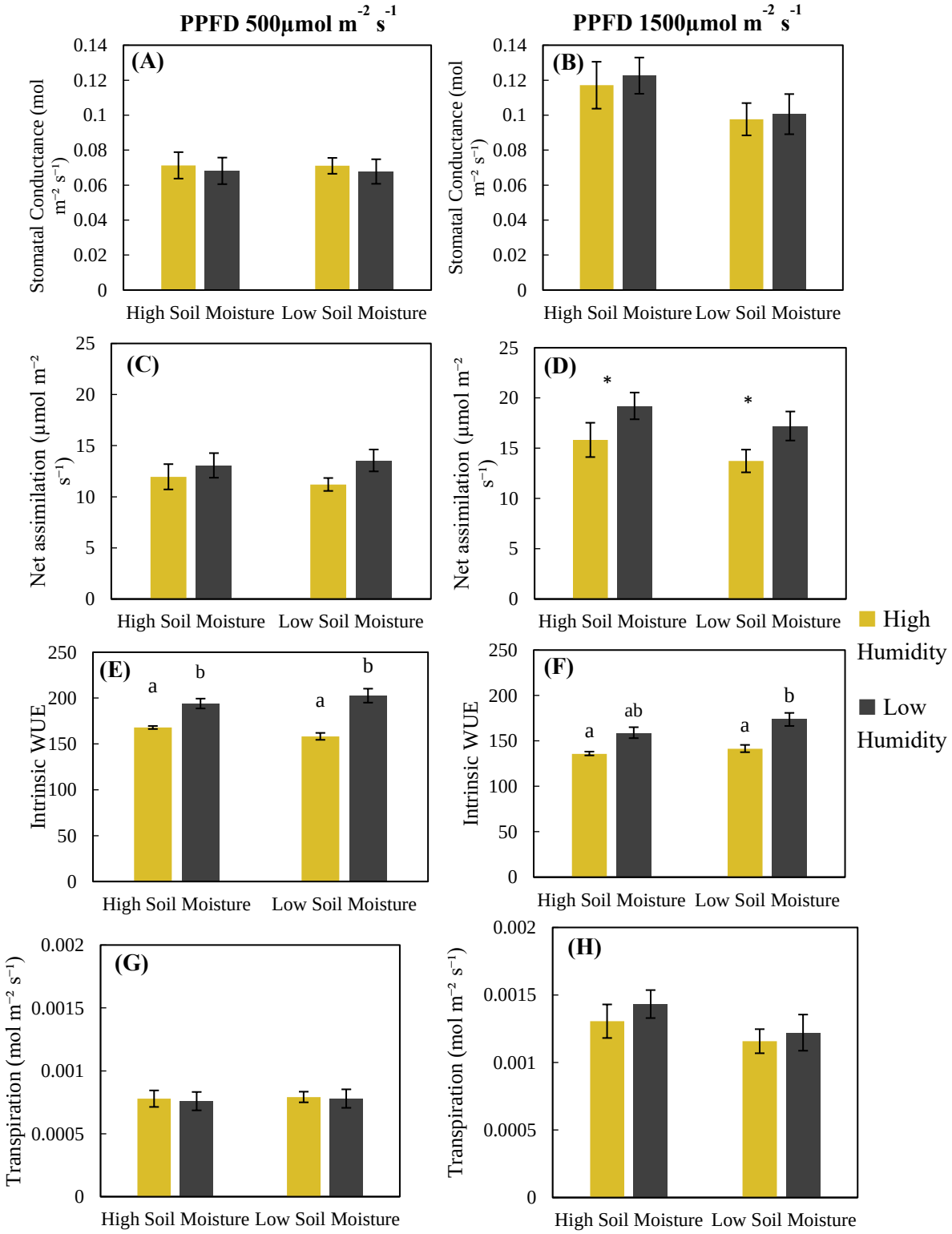


Figure 6. Stomatal conductance (A and B), net assimilation (C and D), intrinsic water use efficiency (E and F), and transpiration (G and H) in maize, under the four treatments: High Humidity High Soil moisture, High Humidity Low Soil moisture, Low Humidity

High Soil moisture and Low Humidity Low Soil moisture Light response measurements were taken at PPFD 500µmol m^{-2} s^{-1} (A,C,E,G) and PPFD 1500µmol m^{-2} s^{-1} (B,D,F,H) on maize plants three weeks after germination. Error bars represent ±SE and different letters represent significance at the 5% level after Tukey test. * represents a significant difference between humidity treatments after general ANOVA ($P<0.05$) n=5.

At PPFD 1500µmol m^{-2} s^{-1}, net assimilation (Figure 6D) for maize was significantly higher under low humidity conditions ($P=0.028$), and maize intrinsic water use efficiency was significantly higher in low humidity conditions, at both PPFD 500µmol m^{-2} s^{-1} (Figure 6E) and 1500µmol m^{-2} s^{-1} (Figure 6F) ($P<0.001$).

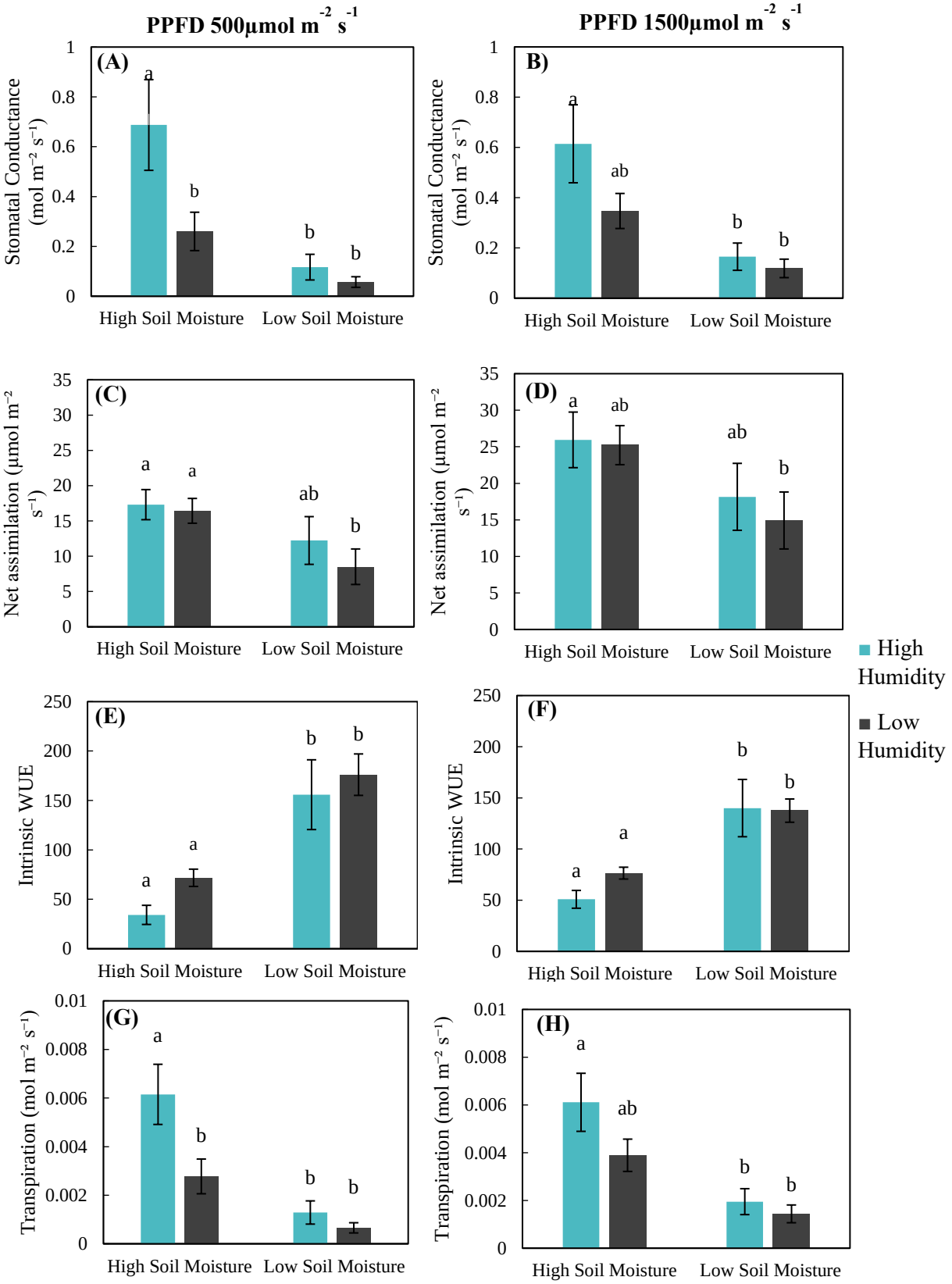


Figure 7. Stomatal conductance (A and B), net assimilation (C and D), intrinsic water use efficiency (E and F), and transpiration (G and H) in wheat under the four treatments:

High Humidity High Soil moisture (HHHS) n=4, High Humidity Low Soil moisture (HHLS) n=5, Low Humidity High Soil moisture (LHHS) n=4 and Low Humidity Low Soil moisture (LHLS) n=5. Light response measurements were taken at PPFD 500µmol m^{-2} s^{-1} (A,C,E,G) and PPFD1500 µmol m^{-2} s^{-1} (B,D,F,H) on wheat plants three weeks after germination. Error bars represent ±SE and different letters represent significance at the 5% level after Fisher's unprotected least significant difference test.

At both PPFD 500 and 1500µmol m^{-2} s^{-1} low soil moisture led to significantly lower stomatal conductance values (P=0.005 and P=0.006) (Figure 7A and B). Under high soil moisture conditions at PPFD 500µmol m^{-2} s^{-1} (Figure 7A), high humidity resulted in significantly greater stomatal conductance (P=0.037).

Wheat net assimilation was significantly higher under high soil moisture conditions when measured with both PPFD 500µmol m^{-2} s^{-1} (Figure 7C) and 1500 (µmol m^{-2} s^{-1}) (Figure 7D) (P=0.023 and P=0.036 respectively). Whereas intrinsic water use efficiency was significantly higher in low soil moisture conditions when measured at both PPFD 500 (Figure 7E) and 1500µmol m^{-2} s^{-1} (Figure 7F) (both P<0.001). Transpiration was significantly higher at high humidity (P=0.034) and high soil moisture (P=0.003) when measured at PPFD 500µmol m^{-2} s^{-1} (Figure 7G), whilst at PPFD 1500µmol m^{-2} s^{-1} (Figure 7H) transpiration was significantly higher in high soil moisture conditions (P=0.004). The HHHS treatment exhibited the highest rates of transpiration across both light levels.

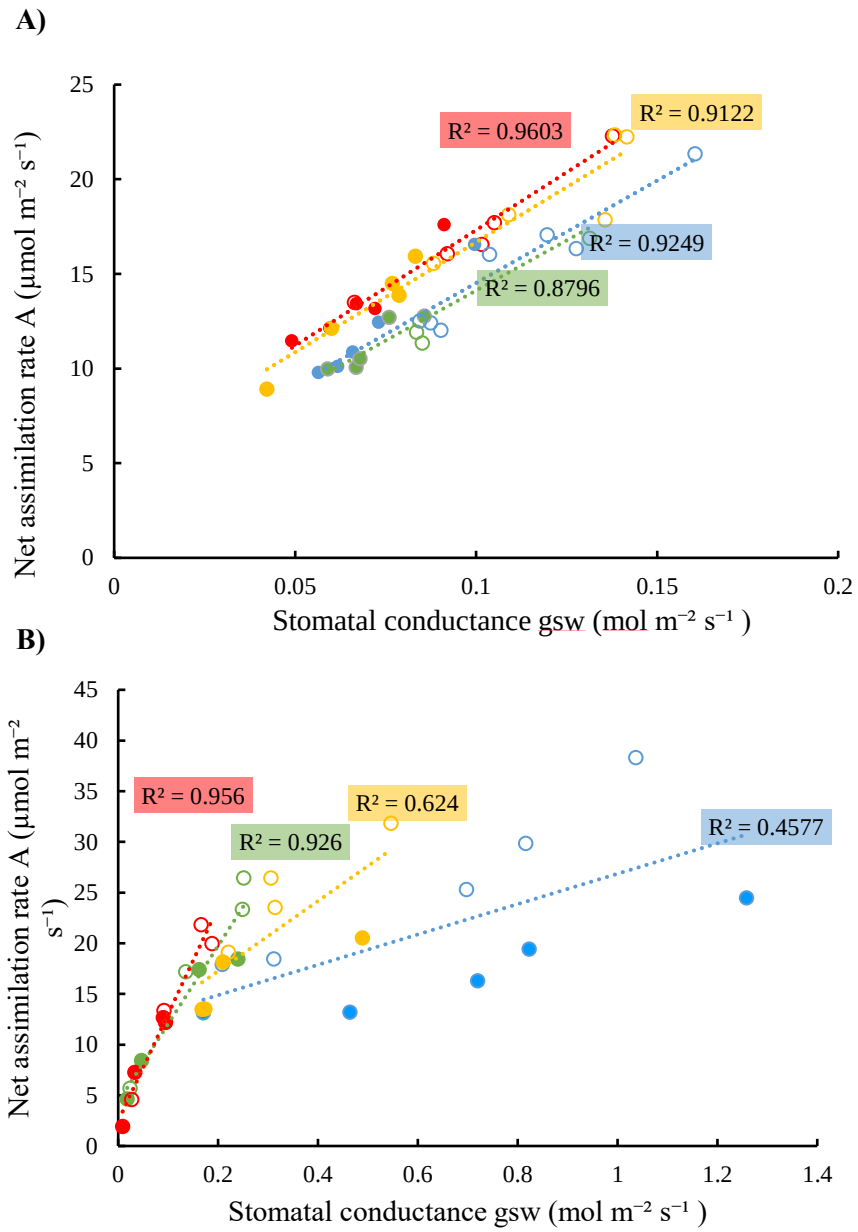


Figure 8. Stomatal conductance vs net assimilation A) maize B) wheat three weeks post-germination. Each colour represents the four treatment conditions, High Humidity High Soil moisture (**HHHS**) (maize n=5 wheat n=4), High Humidity Low Soil moisture (**HHLS**) (maize n=5 wheat n=5), Low Humidity Low Soil moisture (**LHHS**) (maize n=5 wheat n=5) and Low Humidity Low Soil moisture (**LHLS**) (maize n=5 wheat n=5). Linear regression is plotted with a dashed line and corresponding colour to the treatment. Solid filled circles represent spot measurements at PPFD 500µmol m^{-2} s^{-1} and coloured outlined circles represent spot measurements taken at PPFD 1500µmol m^{-2} s^{-1}.

There are positive correlations between stomatal conductance and net assimilation in maize (Figure 8A) the LHLS treatment has the highest net assimilation rate for a given stomatal conductance. The regressions appear to be grouped by humidity with the low humidity treatments (LHHS and LHLS) lying closer together compared to high humidity treatments (HHHS and HHLS) which lie closer to each other. With regards to wheat, the relationships appear to be more strongly affected by soil mositure, as both the low soil moisture treatments have the highest r^2 values (HHLS = 0.93 and LHLS = 0.96). Like maize, the wheat plants in the LHLS treatment have the higher net assimilation rates for a given stomatal conductance.

5.4.3 Chlorophyll Fluorescence

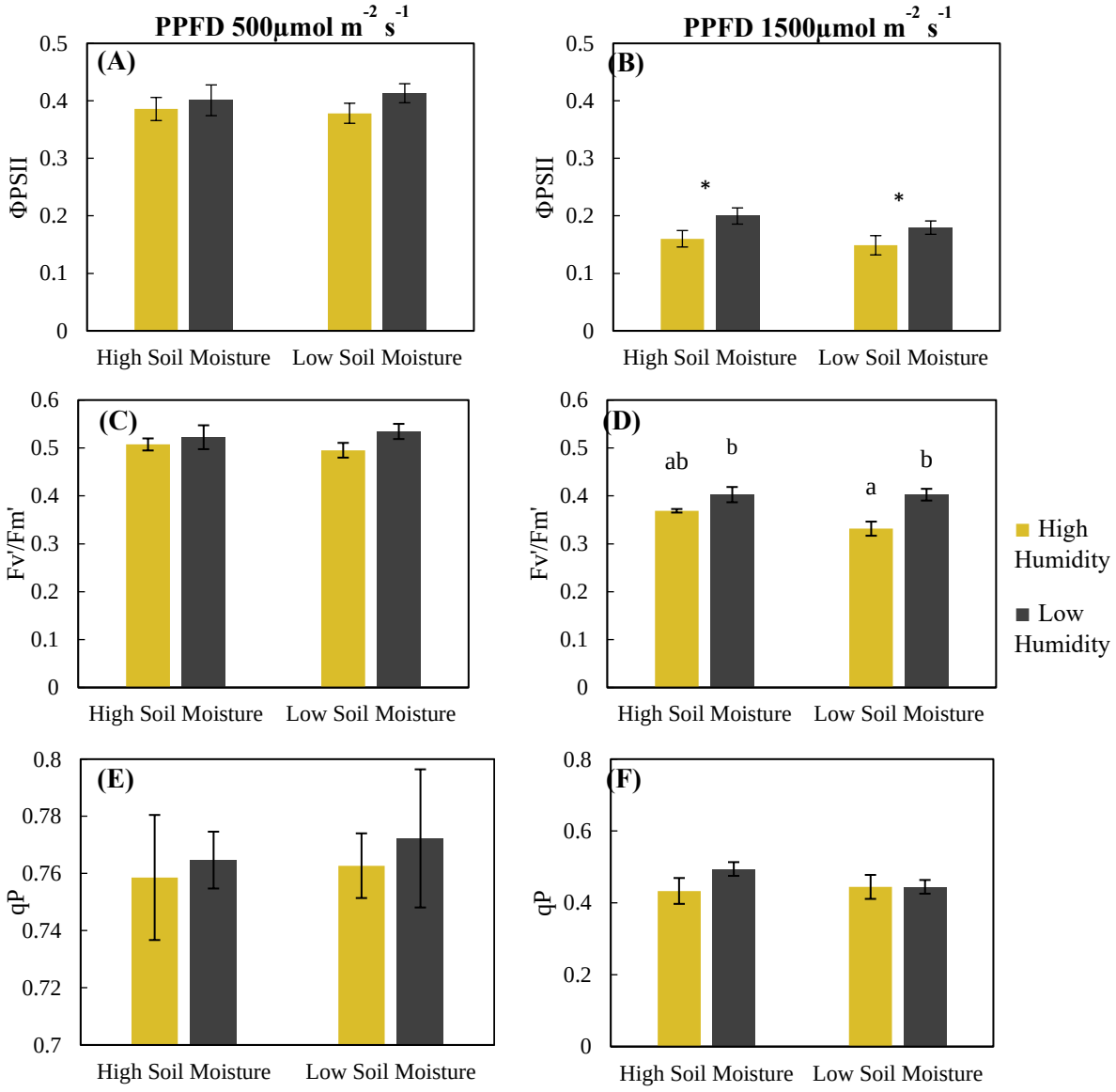


Figure 9. The effects of soil moisture and humidity on ΦPSII (A and B), Fv'/Fm' (C and D) and qP (E and F) in maize under the four treatments: High Humidity High Soil moisture, High Humidity Low Soil moisture, Low Humidity High Soil moisture and Low Humidity Low Soil moisture, three weeks post-germination. Light response measurements were taken at PPFD 500µmol m^{-2} s^{-1} (A,C,E) and PPFD 1500µmol m^{-2} s^{-1} (B,D,F). Error bars represent ±SE, * represents significant humidity main effect, and different letters represent significance at the 5% level after Tukey test, n=5.

ΦPSII (A and B) and qP (E and F) in maize were not significantly affected by soil moisture or humidity, at the 5% level. However, measured Fv'/Fm' at PPFD

1500µmol m^{-2} s^{-1} (D) were significantly affected by humidity (P<0.001). High humidity resulted in significantly lower Fv'/Fm', therefore maximum efficiency of PSII was significantly lower in high humidity conditions, irrespective of soil moisture conditions.

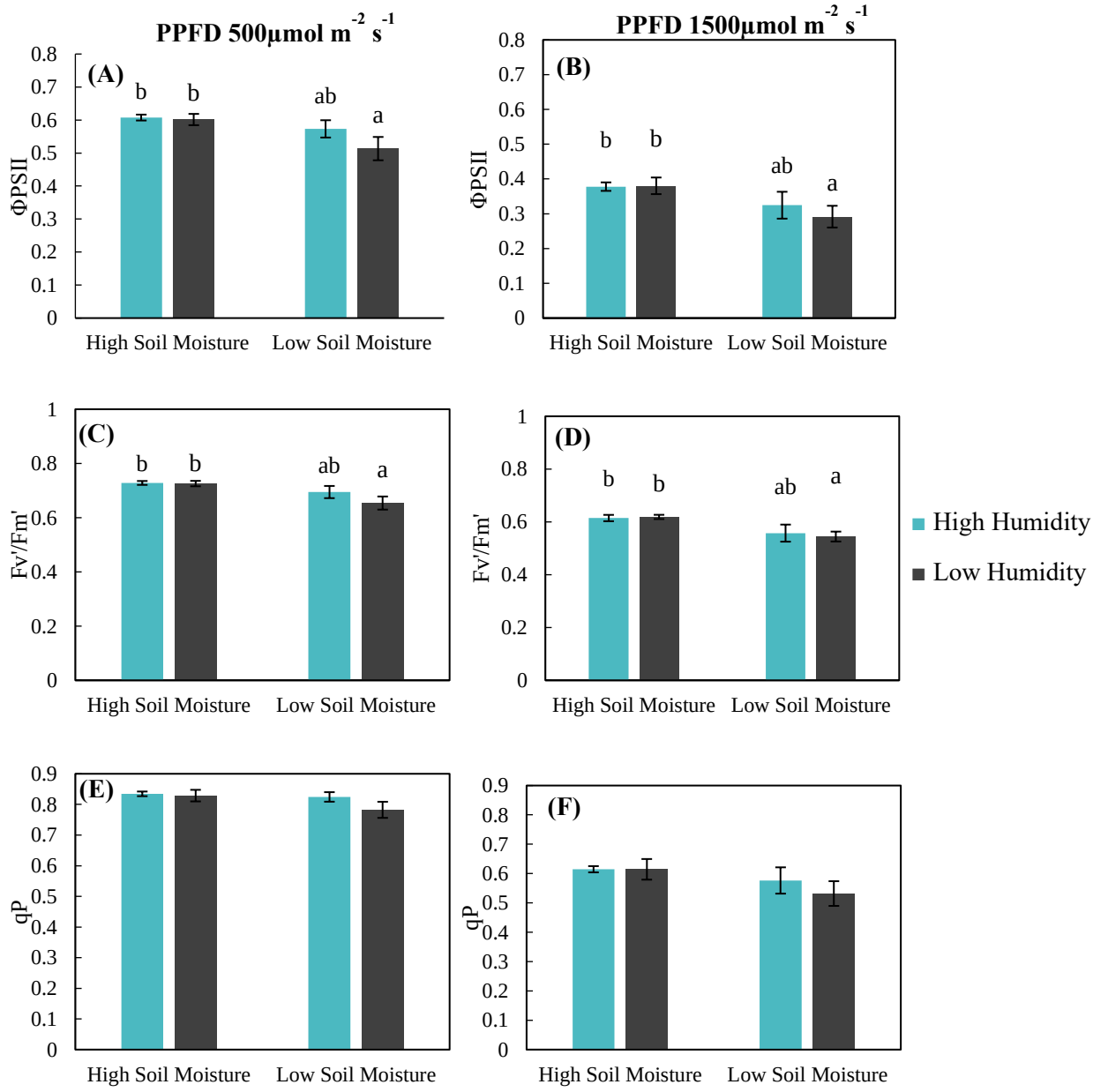


Figure 10. The effects of soil moisture and humidity on ΦPSII (A and B), Fv'/Fm' (C and D) and qP (E and F) under the four treatments: High Humidity High Soil moisture (HHHS), High Humidity Low Soil moisture (HHLS), Low Humidity High Soil moisture (LHHS) and Low Humidity Low Soil moisture (LHLS) in wheat. Light response

measurements were taken at PPFD 500µmol m^{-2} s^{-1} (A,C,D) and PPFD 1500µmol m^{-2} s^{-1} (B,D,F). Error bars represent ±SE and different letters represent significance at the 5% level after post-hoc test Fisher's unprotected least significant difference test. n=5

Wheat ΦPSII (A and B) was significantly affected by soil moisture treatments at both PPFD 500µmol m^{-2} s^{-1} and PPFD 1500µmol m^{-2} s^{-1} (significance for both at P = 0.021). Low soil moisture resulting in significantly lower ΦPSII values, with the lowest values measured under the 1500ppfd light conditions. Fv'/Fm' was also significantly affected by soil moisture as the main effect, at both PPFD 500µmol m^{-2} s^{-1} and PPFD 1500µmol m^{-2} s^{-1} (P = 0.009 and P = 0.005 respectively) (C and D). Low soil moisture results in significantly lower Fv'/Fm' values, regardless of humidity treatment. qP (E and F) was not significantly affected by soil moisture or humidity treatments at the 5% level.

5.4.4 Stomatal Morphology

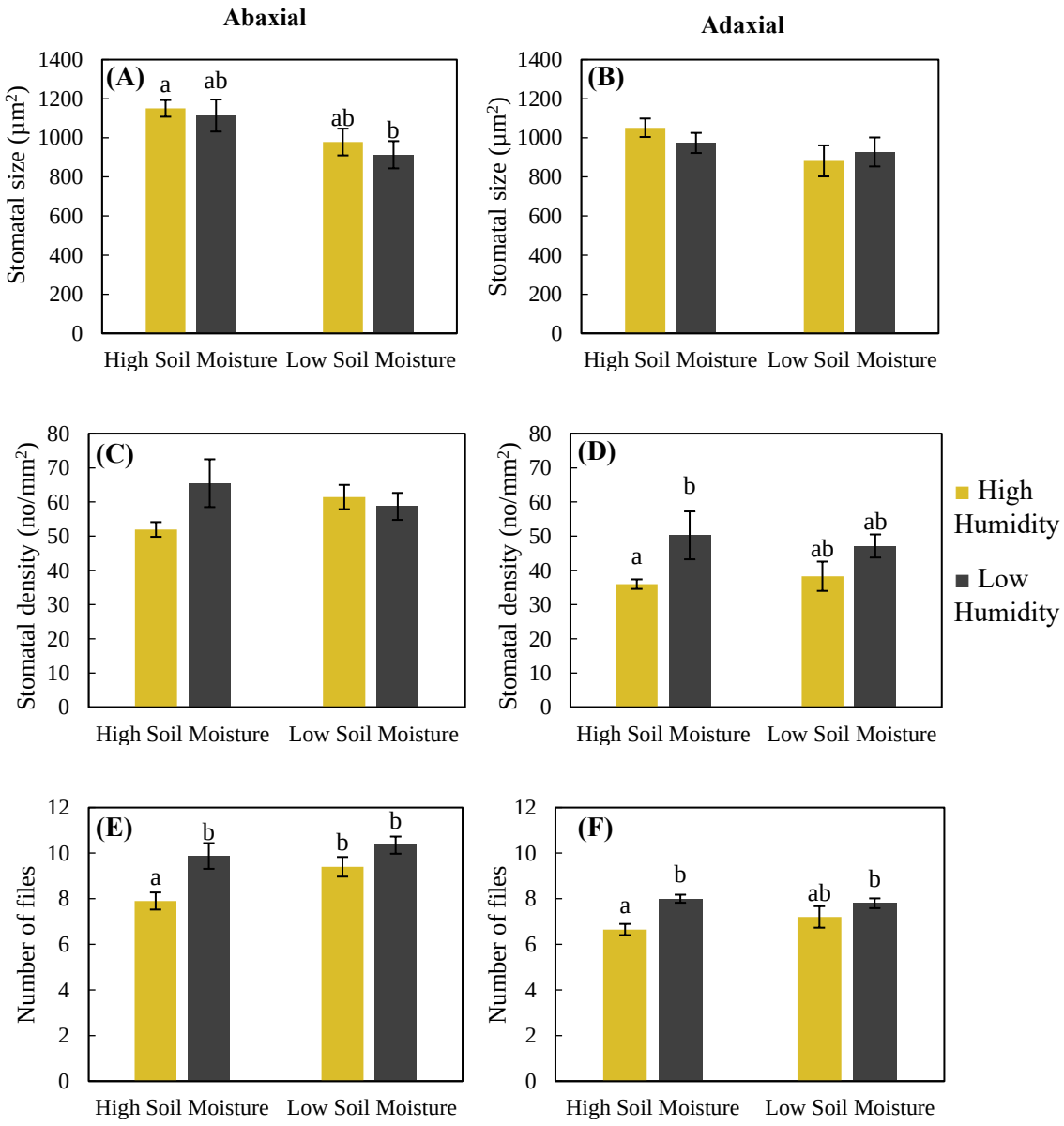


Figure 11. Maize abaxial (left-hand column, A, C, E) and adaxial (right-hand column B, D, F) stomatal morphology of maize plants under the four treatments High Humidity High Soil moisture (n=4), High Humidity Low Soil moisture (n=5), Low Humidity High Soil moisture (n=4), Low Humidity Low Soil Moisture (n=5), measurements made on plants three weeks after germination. Stomatal size (defined here as guard cell length multiplied by the total width of the guard cell pair) (A and B), density (C and D), and the number of files (E and F) are presented. Means are plotted error bars represent ± SE.

Different letters represent significance at the 5% level after post-hoc Fisher's unprotected least significant difference test.

Maize abaxial stomata were significantly larger (Figure 11A) in high soil moisture conditions (P=0.013). Maize adaxial stomatal density (Figure 11D) was significantly affected by humidity (P=0.015), during high soil moisture conditions, high humidity resulted in reduced stomatal density. Similarly, when soil moisture was high, high humidity led to fewer stomatal files (P=0.001).

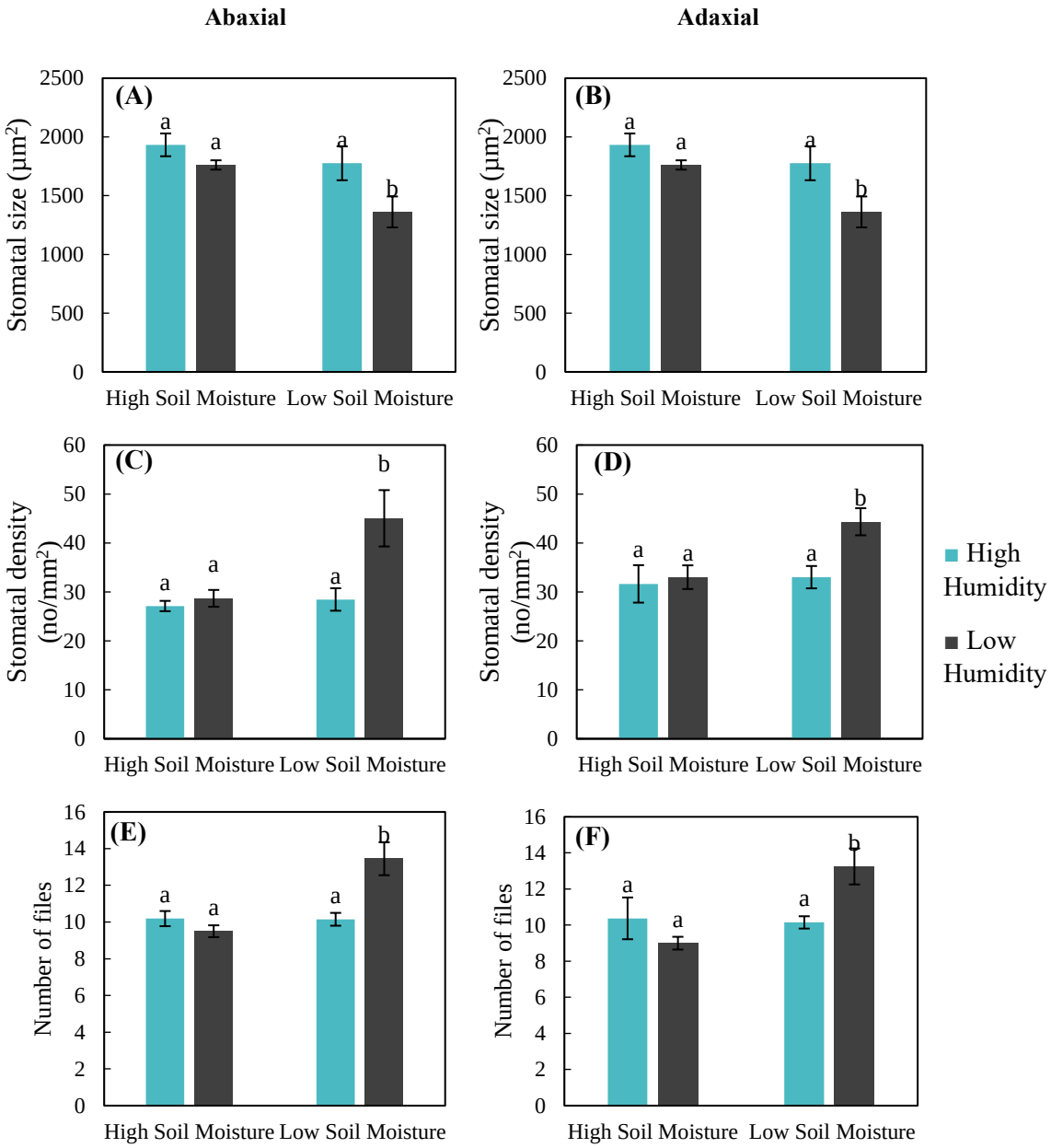


Figure 12. Wheat abaxial (left-hand column, A, C, E) and adaxial (right-hand column B, D, F) stomatal morphology of maize plants in the four treatment conditions: High Humidity High Soil moisture (n=4), High Humidity Low Soil moisture (n=5), Low Humidity High Soil moisture (n=4), Low Humidity Low Soil Moisture (n=5), measurements made on wheat three weeks after germination. Stomatal size (defined here as guard cell length multiplied by the total width of the guard cell pair) (A and B), density (C and D), and the number of files (E and F) are presented. Means are plotted error bars

represent ± SE. Different letters represent significance at the 5% level after post-hoc Fisher's unprotected least significant difference test.

On both abaxial and adaxial leaf surfaces, low humidity and low soil moisture resulted in smaller stomata and under low soil moisture conditions, low humidity led to increased stomatal density and number of files. Wheat abaxial and adaxial stomatal sizes (Figure 12A and B) were affected by humidity (P=0.022 and P=0.026 respectively) and soil moisture (P=0.036 and P=0.022 respectively). Wheat abaxial and adaxial stomatal densities (Figure 12C and D) were affected by humidity (P=0.016 and P=0.028 respectively) and soil moisture (P=0.029 and P=0.042 respectively). The number of files on the abaxial wheat surface (Figure 12E) was significantly affected by humidity, soil moisture, and an interaction between the two (P=0.021, P=0.005, and P=0.005 respectively). Whereas the number of files on the adaxial wheat surface (Figure 12F) was significantly affected by soil moisture and an interaction between humidity and soil moisture (P=0.025 and P=0.014 respectively).

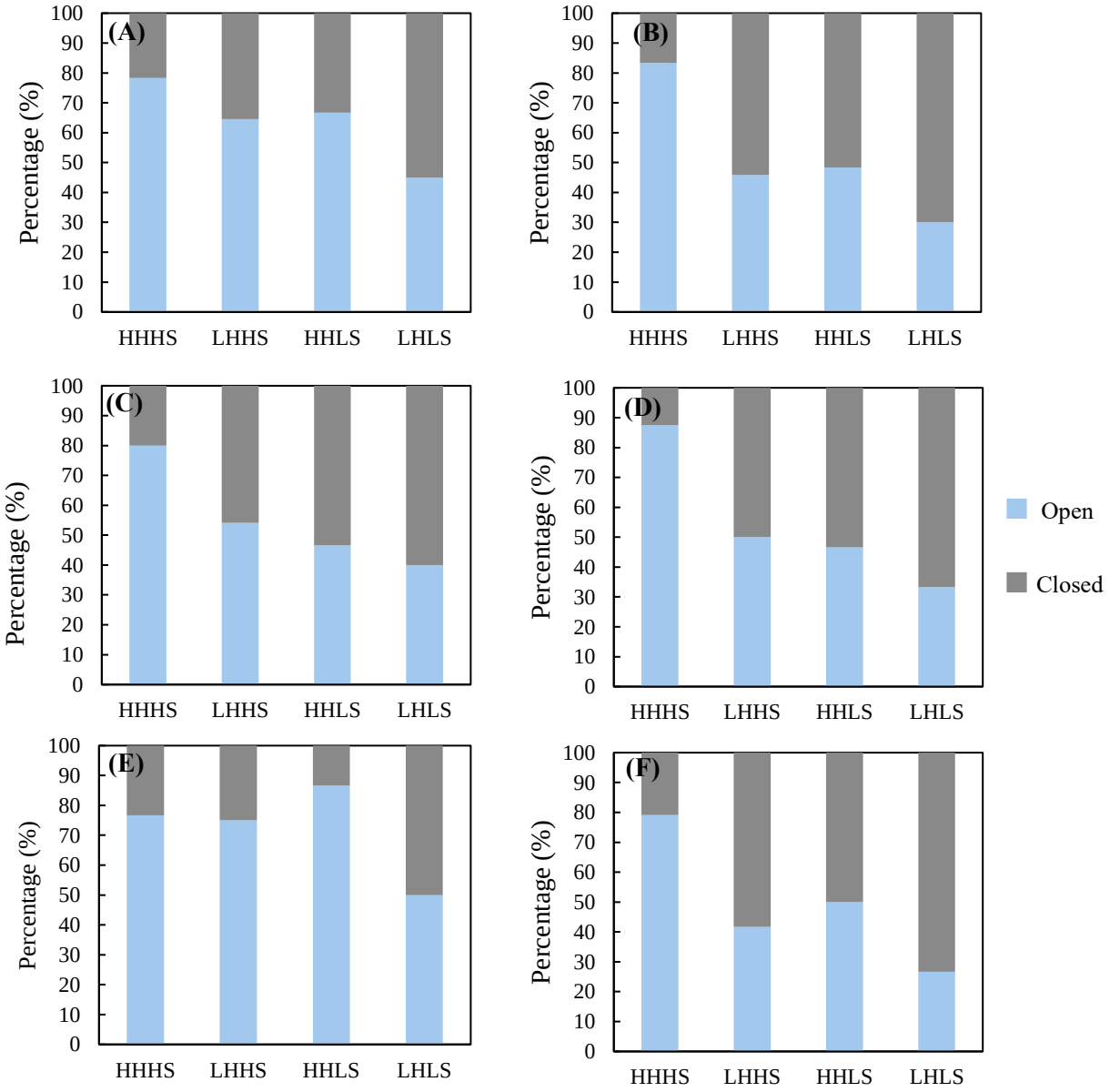


Figure 13. Stomatal aperture for maize the whole leaf average (A), adaxial (C), abaxial (E) and wheat whole leaf average (B), adaxial (D) and abaxial (F) sides of the leaf, in response to treatment conditions: High Humidity High Soil moisture (HHHS) maize n=5 wheat n=4, Low Humidity High Soil Moisture (LHHS) maize n=5, wheat n=4, High Humidity Low Soil Moisture (HHLS) maize n=4, wheat n=5 and Low Humidity Low Soil Moisture (LHLS) maize n=5 wheat n=5. Three weeks after germination. The significant interaction of treatments on the proportion of open/closed stomata calculated a Chi-squared significance test at the 5% level, on maize count data (A) P=0.013, (C) P=0.011, and (E) P=0.002 and wheat (B) P<0.001, (D) P<0.001 and (F) P=0.002.

The treatment conditions significantly affected the proportion of open and closed maize stomata on the whole leaf (Figure 13A), adaxial (Figure 13C), and abaxial (Figure 13E), surface ($P=0.013$, $P=0.011$, and $P=0.002$ respectively). The whole leaf response (Figure 13A) showed stomata remained predominantly open in all treatments except the low humidity low soil moisture (LHLS) where more than 50% were closed. There appeared to be differences between abaxial and adaxial responses, where abaxial stomata remained predominantly open across most treatments regardless of humidity or soil moisture, only showing considerable closure when both humidity and soil moisture was low (LHLS). Though adaxial stomata appear to show a greater proportion of closure than abaxial when humidity and/or soil moisture was low.

The treatment conditions significantly affected the proportion of open and closed wheat stomata on the whole leaf (Figure 13B), adaxial (Figure 13D), and abaxial (Figure 13F), surface ($P<0.001$, $P<0.001$ and $P=0.002$, respectively). Unlike maize, both sides of the wheat leaf appeared to respond to treatment conditions in a similar fashion. Low soil moisture and/or low humidity-induced stomatal closure. A vast majority of stomata remained open in the HHHS treatment.

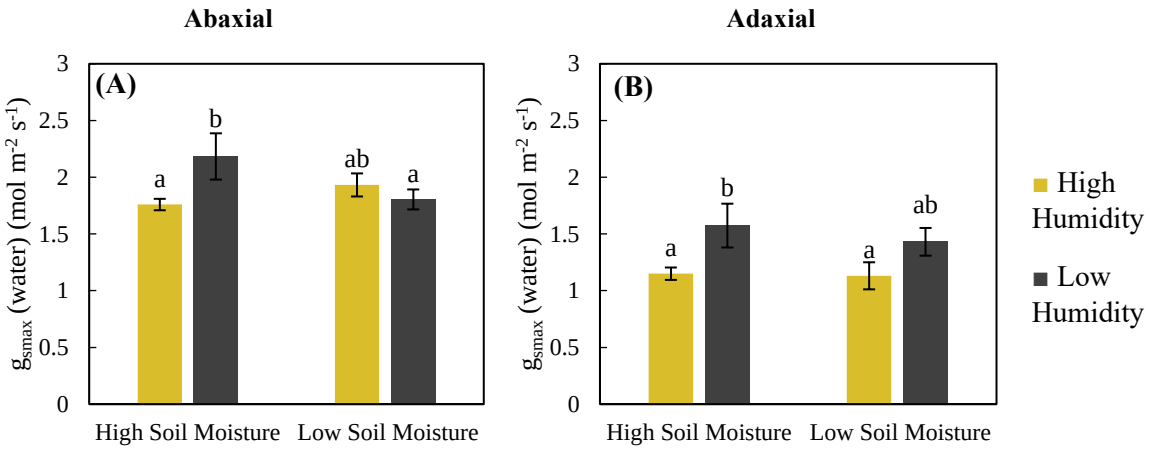


Figure 14. Maize abaxial (A) and adaxial (B) maximum stomatal conductance (g_{smax}) to water in response to the four treatment conditions, High Humidity High Soil moisture (n=5), High Humidity Low Soil moisture (n=5), Low Humidity High Soil moisture (n=4), Low Humidity Low Soil Moisture (n=5), three weeks post-germination. g_{smax} was calculated as in Franks and Beerling (2009). Means are plotted error bars represent ± SE. Different letters represent significance at the 5% level after post-hoc Fisher's unprotected least significant difference test.

Maize abaxial g_{smax} (Figure 14A) was significantly affected by humidity soil moisture interaction (P=0.028). During high soil moisture conditions, high humidity significantly reduced g_{smax}, whereas, during low humidity conditions, low soil moisture significantly reduces g_{smax}. Maize adaxial g_{smax} (Figure 14B) was significantly lower under high humidity conditions (P=0.012) regardless of soil moisture conditions.

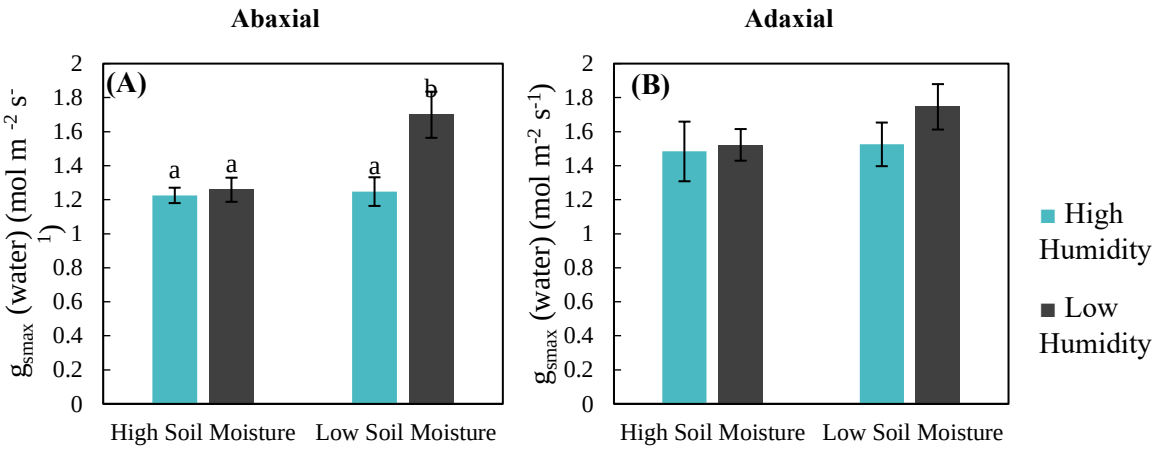


Figure 15. Wheat abaxial (A) and adaxial (B) g_{smax} responses to treatment conditions: High Humidity High Soil moisture (n=4), High Humidity Low Soil moisture (n=5), Low Humidity High Soil moisture (n=4), Low Humidity Low Soil Moisture (n=5). g_{smax} was calculated as in Franks and Beerling (2009), on wheat three weeks after germination. Means are plotted error bars represent ± SE. Different letters represent significance at the 5% level after post-hoc Fisher's unprotected least significant difference test.

Abaxial g_{smax} (Figure 15A) was significantly affected by humidity and soil moisture (P=0.016 and P=0.033 respectively). When soil moisture was low, low humidity led to significantly higher abaxial g_{smax}, whereas when humidity was low, low soil moisture led to significantly higher abaxial g_{smax}.

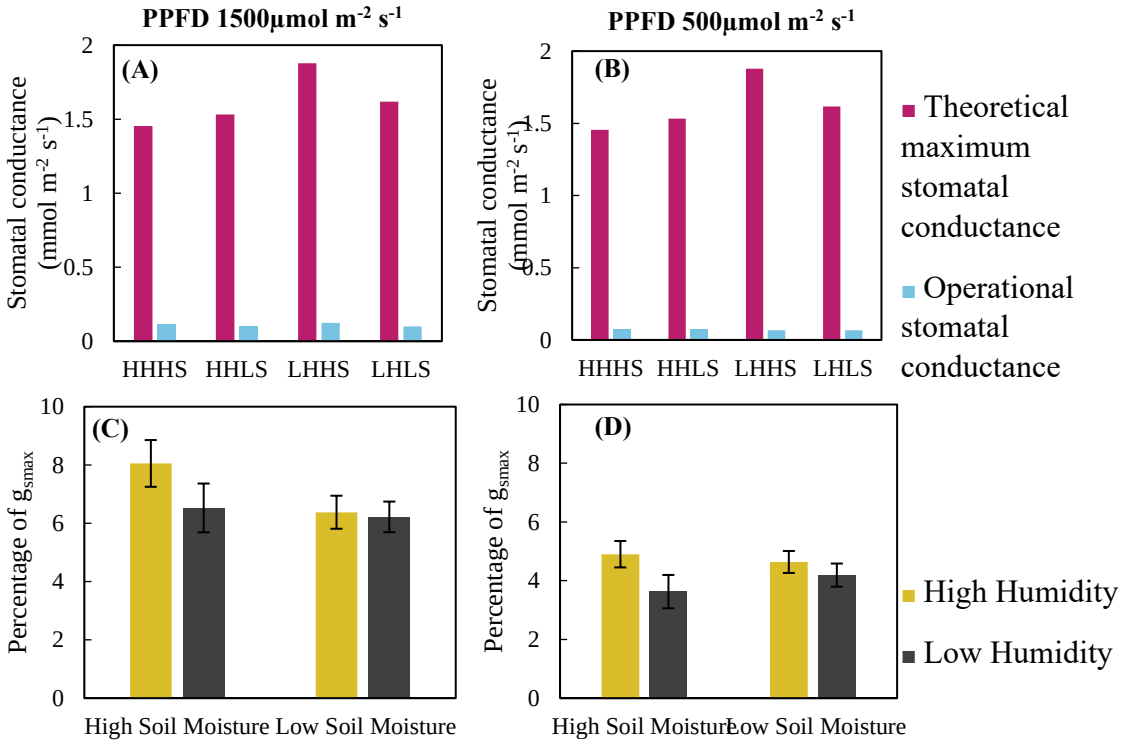


Figure 16. The calculated theoretical maximum stomatal conductance (g_{smax}) and measured operational stomatal conductance (g_s) values for maize at PPFD 1500µmol m^{-2} s^{-1} (A), and PPFD 500µmol m^{-2} s^{-1} (B). The percentage difference between gsmax and gs is plotted for 1500 µmol m^{-2} s^{-1} measurements (C) and 500 µmol m^{-2} s^{-1} (D). Across treatment conditions, High Humidity High Soil moisture (HHHS) n=5, High Humidity Low Soil moisture (HHLS) n=5, Low Humidity High Soil moisture (LHHS) n=4, Low Humidity Low Soil moisture (LHLS) n=5, in maize three weeks post-germination. Means are plotted, error bars represent ±SE.

Throughout all treatments maize operational stomatal conductance did not reach above 9% of the theoretical maximum at the highest light levels (PPFD 1500µmol m^{-2}). In contrast wheat performed closer to the theoretical maximum across all treatments when compared to maize.

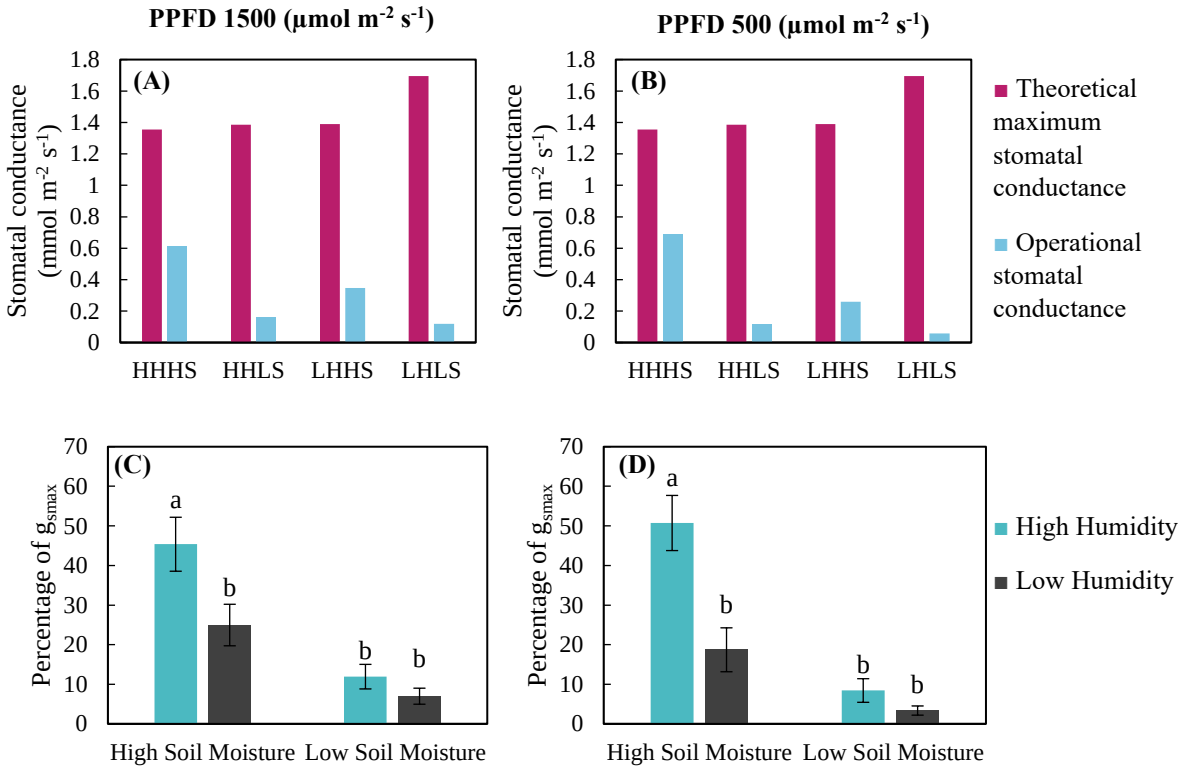


Figure 17. Calculated theoretical maximum stomatal conductance (g_{smax}) as in Franks and Beerling (2009), and measured operational stomatal conductance (g_s) values for wheat at PPFD 1500 µmol m^{-2} s^{-1} (A), and PPFD 500 µmol m^{-2} s^{-1} (B). The percentage difference between g_{smax} and g_s is plotted for PPFD 1500 µmol m^{-2} s^{-1} measurements (C) and PPFD 500 µmol m^{-2} s^{-1} (D). Across the four treatment conditions, High Humidity High Soil moisture (HHHS) n=4, High Humidity Low Soil moisture (HHLS) n=5, Low Humidity High Soil moisture (LHHS) n=4, and Low Humidity Low Soil moisture (LHLS) Means are plotted, error bars represent ±SE. Different letters represent significance at the 5% level after post-hoc Fisher's unprotected least significant difference test.

The proportion of the theoretical maximum stomatal conductance that was achieved under both (Figure 17C) 1500 and (Figure 17D) PPFD 500µmol m^{-2} s^{-1} was significantly affected by humidity (P=0.018 and P=0.002) respectively, and soil moisture (P<0.001 and P=0.008). As well as a significant interaction between the two at 500µmol m^{-2} s^{-1} (P=0.008). Wheat grown under the HHHS treatment

reached approximately 50% of its theoretical maximum, a significantly higher percentage than all other treatments.

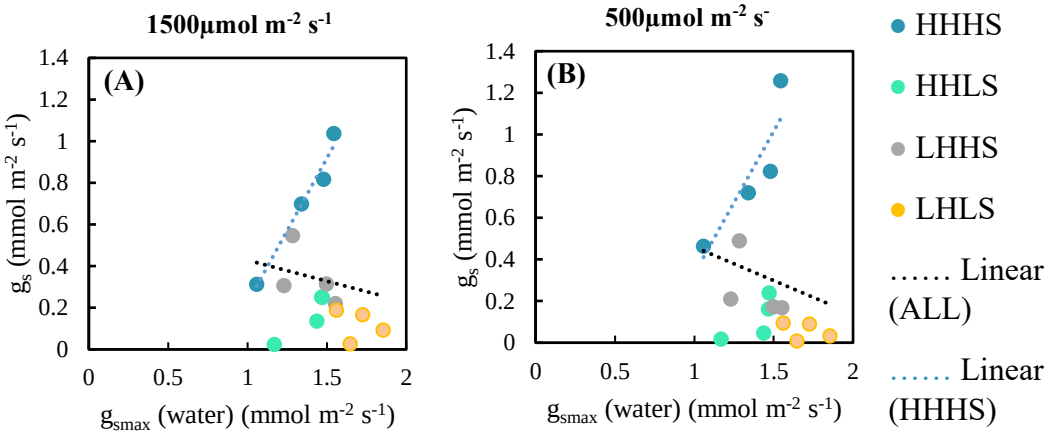


Figure 18. g_{smax} vs g_s in wheat measured at (A) PPFD 1500µmol m^{-2} s^{-1} and (B) 500µmol m^{-2} s^{-1} Linear regression carried out on entire all data points, and r^2 values are presented on the graph. For (A) and (B), an additional linear regression represents the strong positive correlation between g_{smax} and g_s in wheat grown in high humidity high soil moisture (HHHS) conditions. n=4 per treatment, n=16 for linear ALL. r^2 values are (A) 0.9686 (HHHS) and 0.0202 (ALL), and (B) 0.7907 (HHHS) and 0.0351 (ALL).

There is a strong positive correlation between g_{smax} and g_s in wheat plants grown under HHHS treatment conditions (r^2=0.97).

5.4.5 Biomass

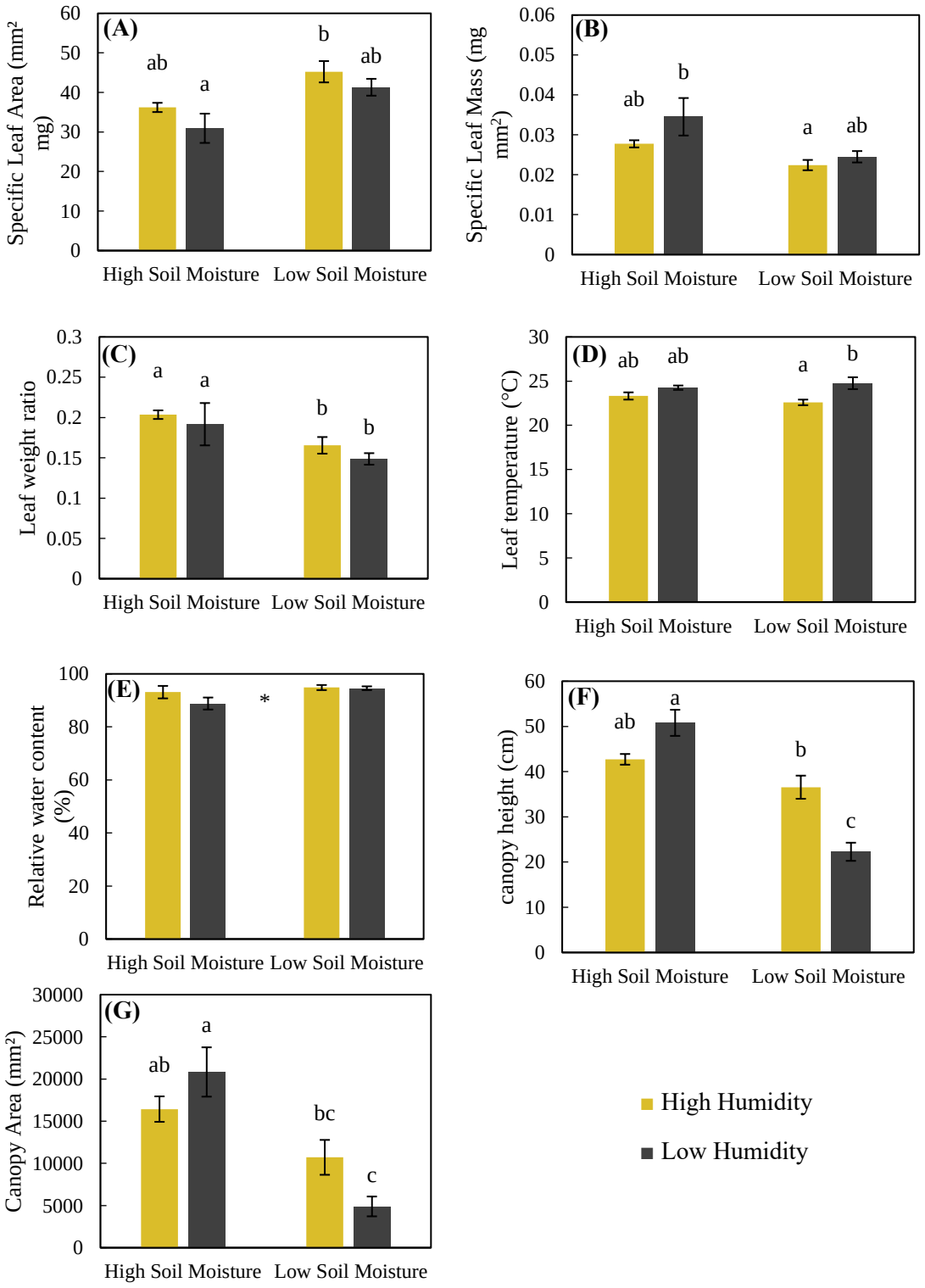


Figure 19. The effects of soil moisture and humidity on maize (A) specific leaf area, (B) specific leaf mass, (C) leaf weight ratio (thickness), (D), leaf temperature, (E) relative water content, (F) canopy height, and (G) canopy area, under the four treatments: High

Humidity High Soil moisture, High Humidity Low Soil moisture, Low Humidity High Soil moisture and Low Humidity Low Soil moisture, three weeks post-germination. Error bars represent ±SE, * represents significant soil moisture treatment effect and different letters represent significance at the 5% level after post-hoc Tukey test, n=5.

Maize specific leaf area (Figure 19A) and relative water content (Figure 19E) were significantly higher in low soil moisture conditions (P=0.002 and P=0.046 respectively), regardless of humidity. Whereas specific leaf mass (Figure 19B) and leaf weight ratio (Figure 19C), were both significantly lower during low soil moisture conditions (P=0.009 and P=0.015 respectively). Whilst leaf temperature (Figure 19D) was significantly reduced during high humidity conditions (P=0.003).

At the canopy level, height (Figure 19F) and area (Figure 19G) were affected by both soil moisture (P<0.001 for both) and a soil moisture humidity interaction (P<0.001 and P=0.023 respectively). During high soil moisture conditions, high humidity led to a reduced canopy height and area, in contrast, under low soil moisture conditions, high humidity led to increased canopy height and area.

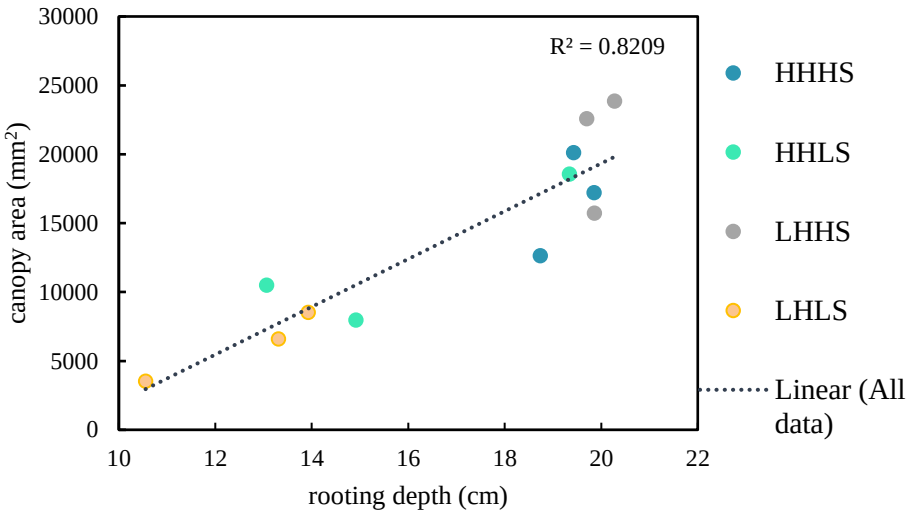


Figure 20. The positive relationship between maize rooting depth and canopy area (mm^2) ($r^2 = 0.82$) in maize plants three weeks after germination. Grown under the following treatment conditions: High Humidity High Soil Moisture (HHHS), High Humidity Low Soil Moisture (HHLS), Low Humidity High Soil Moisture (LHHS), and Low Humidity Low Soil Moisture (LHLS). A linear trendline is plotted, based on all data n=12.

There is a strong positive relationship between maize rooting depth and the area of the canopy (r^2=0.82).

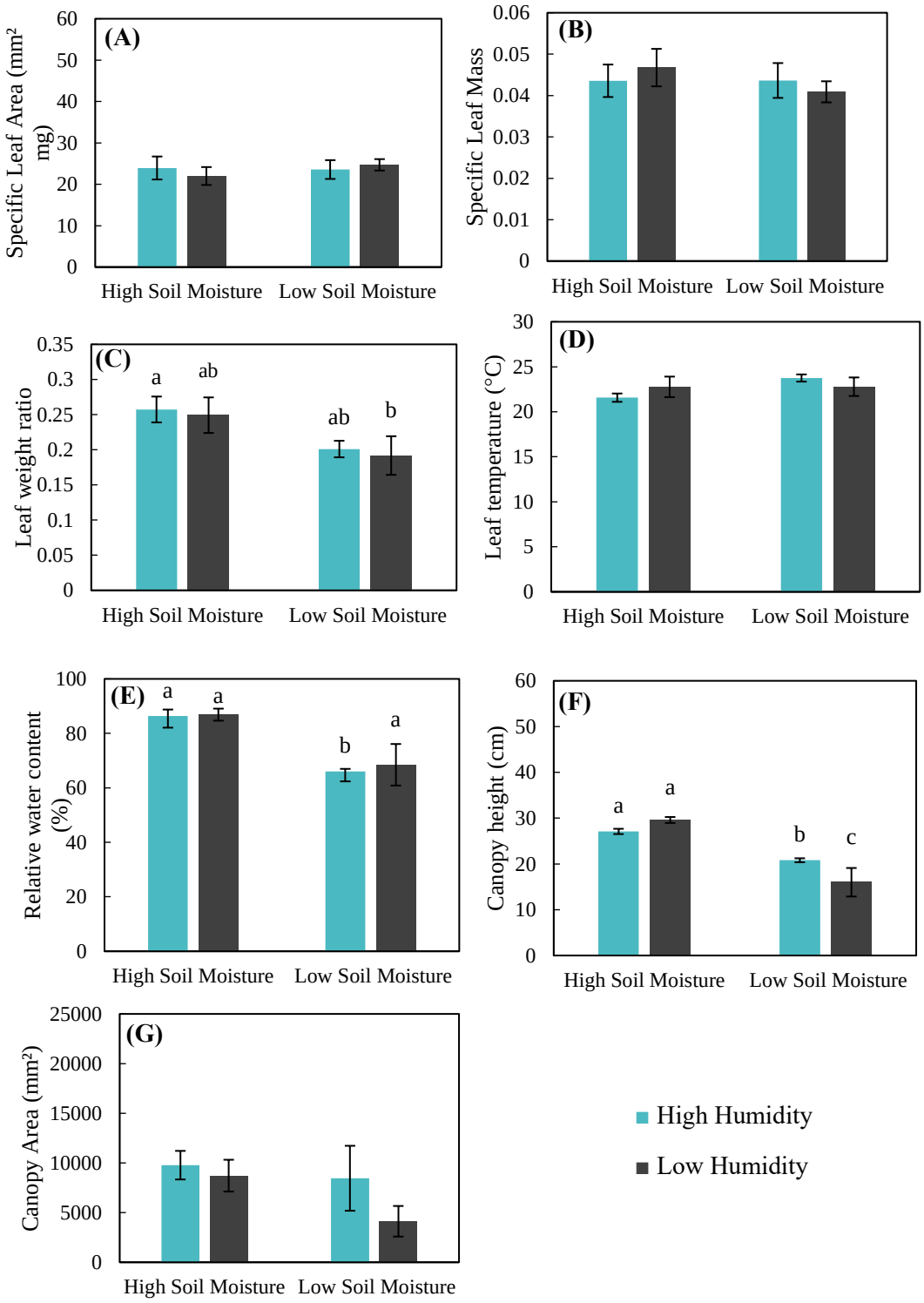


Figure 21. The effect of soil moisture and humidity on wheat (A) specific leaf area, (B) specific leaf mass, (C) leaf weight ratio (thickness), (D), leaf temperature, (E) relative water content, (F) canopy height, and (G) canopy area, under the four treatments: High Humidity High Soil moisture (n=4), High Humidity Low Soil moisture (n=5), Low Humidity High Soil moisture (n=4) and Low Humidity Low Soil moisture (n=5), three

weeks post-germination. Error bars represent ±SE, different letters represent significance at the 5% level after post-hoc Tukey test.

Wheat was less responsive when compared to maize although some significant effects are detected. The wheat leaf weight ratio (Figure 21C) and relative water content (Figure 21E) were both lower in low soil moisture conditions ($P=0.02$ and $P=0.001$ respectively). Furthermore, the canopy height was reduced under low soil moisture conditions ($P<0.001$), and further reduced when humidity was low (Figure 21F) ($P=0.034$).

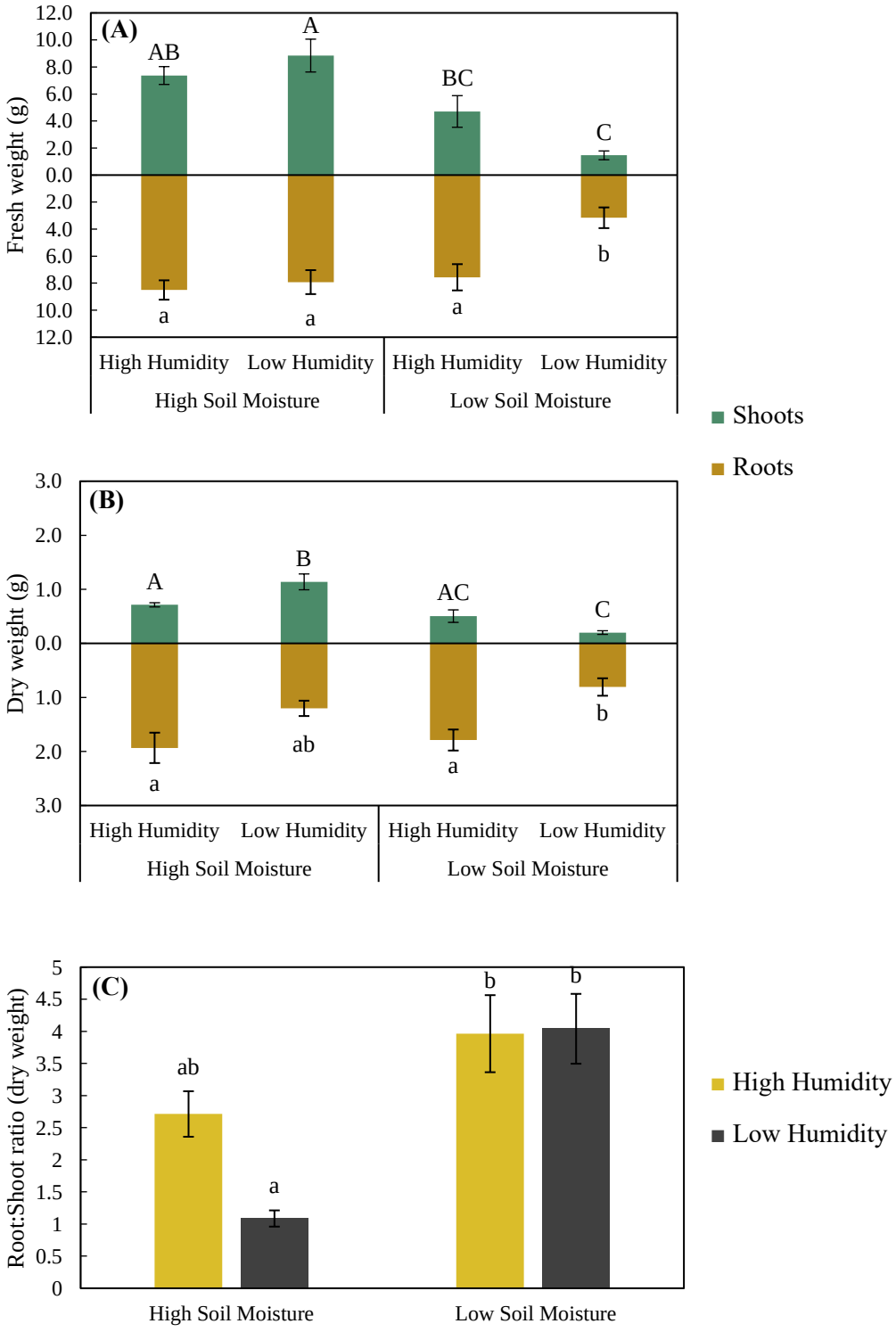


Figure 22. Maize biomass responses of (A) shoot and root fresh weights, (B) shoot and root dry weights, and (C) root:shoot ratio (dry weight) to treatment conditions (High Humidity High Soil Moisture, Low Humidity High Soil Moisture, High Humidity Low Soil Moisture, and Low Humidity Low Soil Moisture, three weeks post-germination. Values presented are mean values ± SE. Different letters represent significance at the 5%

level after post-hoc Tukey test. With regards to (A) and (B), uppercase letters represent a significant difference between treatments in the shoots, whilst lowercase letters represent a significant difference between treatments in the roots. n=5.

Maize shoot fresh weight (Figure 22A) was significantly higher in high soil moisture conditions ($P = <0.001$). Whereas maize root fresh weight was significantly affected by both soil moisture and humidity ($P = 0.004$ and $P = 0.009$ respectively) as well as a significant interaction between soil moisture and humidity ($P = 0.037$), with lower root fresh weights in the LHLS treatment.

With regards to dry weight (Figure 22B), maize shoot dry weight was significantly affected by soil moisture ($P < 0.001$) as well as a significant interaction between soil moisture and humidity ($P = 0.002$). Under low humidity conditions, high soil moisture resulted in higher shoot dry weight, whereas under high soil moisture condition, low humidity led to higher shoot dry weights, significantly higher than all other treatments. Unlike the shoots, maize root dry weights were significantly affected by humidity ($P < 0.001$) as high humidity led to significantly greater root dry weights. The maize root:shoot ratio (Figure 22C) was significantly affected by soil moisture as the main effect ($P<0.001$), as low soil moisture resulted in a higher root:shoot, regardless of humidity treatment conditions.

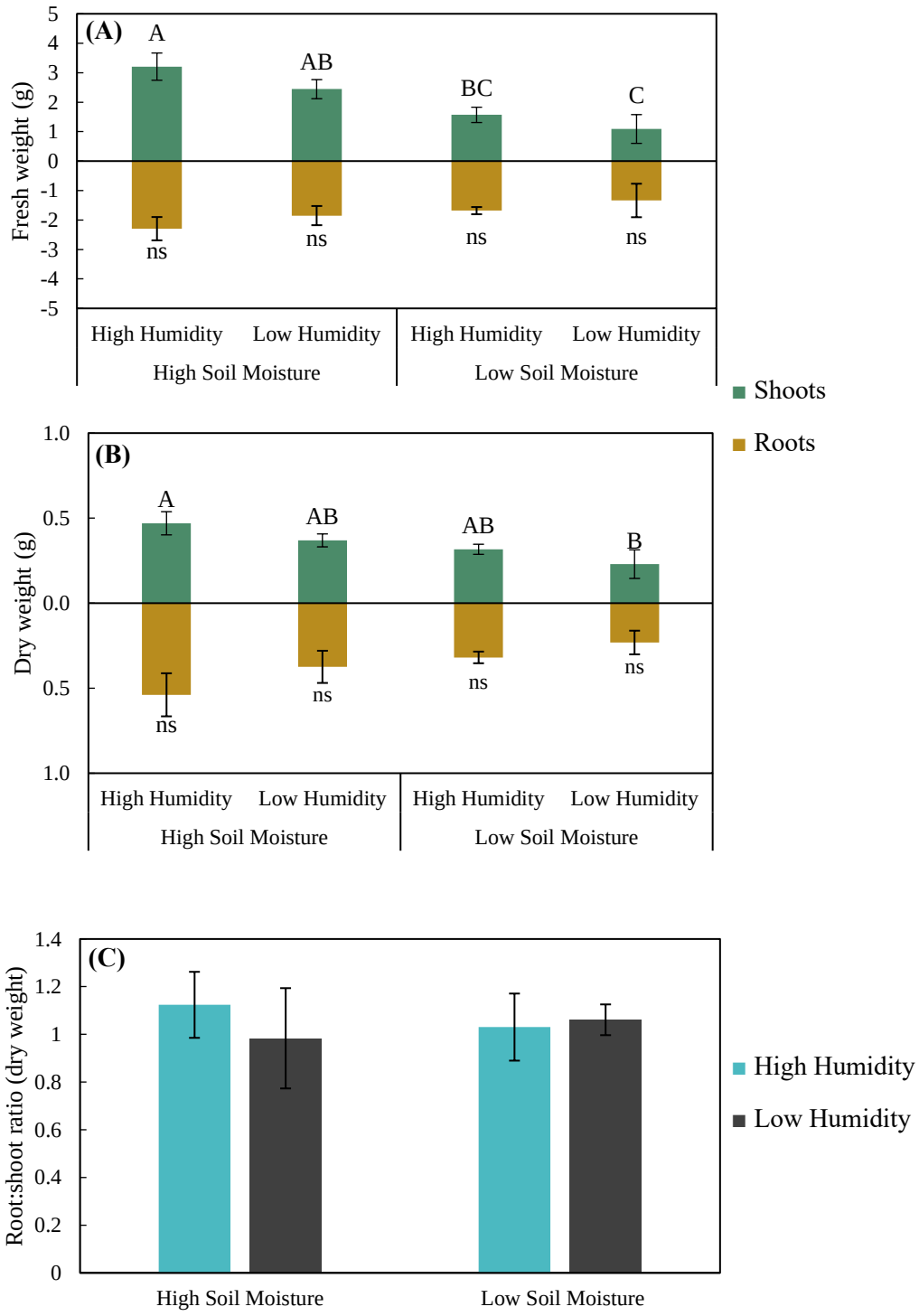


Figure 23. Wheat biomass responses of (A) shoot and root fresh weights, (B) shoot and root dry weights, and (C) root:shoot ratio (dry weight) to the treatment conditions, High Humidity High Soil Moisture (n=4), High Humidity Low Soil moisture (n=5), Low Humidity High Soil moisture (n=4), and Low Humidity Low Soil moisture (n=5), three

weeks post-germination. Values presented are mean values ± SE. Different letters represent significance at the 5% level after post-hoc Tukey test. With regards to (A) and (B), uppercase letters represent a significant difference between treatments in the shoots, whilst lowercase letters represent a significant difference between treatments in the roots. ns represent no significant difference.

Wheat shoot fresh weight (Figure 23A) was significantly affected by soil moisture as well as a significant interaction between soil moisture and humidity ($P<0.001$ and $P=0.021$ respectively). Within the high humidity treatment, high soil moisture led to significantly higher shoot fresh weights, the same was witnessed in the low humidity treatment, as shoot fresh weight significantly increased in high soil moisture. Wheat shoot dry weights were significantly affected by soil moisture ($P = 0.032$) with the lowest shoot dry weights from plants grown in low soil moisture conditions and low humidity (Figure 23B). Unlike the shoots, there were no significant treatment effects on root fresh and dry weight.

Table 2 A table summarising the statistical output from General ANOVAs carried out throughout this chapter on maize and wheat plant physiology. Shaded boxes (■) represent significance at the 5% level.

		Maize			Wheat		
Figure	Panel	Humidity	Soil moisture	Humidity × soil moisture interaction	Humidity	Soil moisture	Humidity × soil moisture interaction
Root architecture Figure 2 and Figure 4	(A) Rooting depth	0.269	<0.001	0.119	0.313	<.001	0.284
	(B) Root volume	0.022	0.069	0.015	0.251	0.480	0.545
	(C) Root surface area	0.020	0.036	0.016	0.463	0.739	0.671
	(D) Root surface area : volume ratio	0.592	0.931	0.461	0.030	0.078	0.764
	(E) Total root length	0.048	0.041	0.011	0.253	0.319	0.926
Gas exchange Figure 6 and Figure 7	(A) Stomatal conductance (500µmol m^{-2} s^{-1})	0.645	0.963	0.993	0.037	0.005	0.146
	(B) Stomatal conductance (1500 µmol m^{-2} s^{-1})	0.714	0.083	0.911	0.103	0.006	0.308
	(C) Net assimilation (500µmol m^{-2} s^{-1})	0.123	0.903	0.569	0.319	0.023	0.579
	(D) Net assimilation (1500µmol m^{-2} s^{-1})	0.028	0.168	0.973	0.540	0.036	0.749
	(E) Intrinsic water use efficiency (500µmol m^{-2} s^{-1})	<.001	0.926	0.129	0.154	<.001	0.721
	(F) Intrinsic water use efficiency (1500µmol m^{-2} s^{-1})	<.001	0.094	0.417	0.365	<.001	0.440
	(G) Transpiration (500µmol m^{-2} s^{-1})	0.829	0.822	0.962	0.034	0.003	0.178
	(H) Transpiration (1500µmol m^{-2} s^{-1})	0.454	0.166	0.801	0.122	0.004	0.394
Chlorophyll fluorescence Figure 9 and Figure 10	(A) ΦPSII (500 µmol m^{-2} s^{-1})	0.241	0.908	0.638	0.149	0.021	0.259
	(B) ΦPSII (1500µmol m^{-2} s^{-1})	0.026	0.286	0.759	0.507	0.021	0.523
	(C) Fv'/Fm' (500µmol m^{-2} s^{-1})	0.147	0.998	0.506	0.186	0.009	0.274
	(D) Fv'/Fm'(1500µmol m^{-2} s^{-1})	<.001	0.156	0.159	0.701	0.005	0.663
	(E) qP (500µmol m^{-2} s^{-1})	0.631	0.719	0.915	0.183	0.142	0.316
	(F) qP (1500µmol m^{-2} s^{-1})	0.291	0.503	0.293	0.479	0.102	0.525
Stomatal morphology Figure 11 and Figure 12	(A) Stomatal size (abaxial)	0.362	0.013	0.833	0.022	0.036	0.328
	(B) Stomatal size (adaxial)	0.784	0.114	0.368	0.026	0.022	0.131
	(C) Stomatal density (abaxial)	0.249	0.67	0.071	0.016	0.029	0.057

	(D) Stomatal density (adaxial)	0.015	0.956	0.525		0.028	0.042	0.102
	(E) Number of stomatal files (abaxial)	0.004	0.032	0.254		0.021	0.005	0.005
	(F) Number of stomatal files (adaxial)	0.008	0.538	0.25		0.184	0.025	0.014
Canopy Figure 19 and Figure 21	(A) Specific leaf area	0.095	0.002	0.797		0.859	0.649	0.520
	(B) Specific leaf mass	0.105	0.009	0.376		0.955	0.498	0.464
	(C) Leaf weight ratio	0.347	0.015	0.867		0.590	0.020	0.980
	(D) Leaf temperature	0.003	0.806	0.182		0.768	0.168	0.195
	(E) Relative water content	0.208	0.046	0.263		0.977	0.001	0.836
	(F) Canopy height	0.731	<.001	0.023		0.198	0.183	0.437
	(G) Canopy area	0.731	<.001	0.023		0.198	0.183	0.437
Biomass Figure 22 and Figure 23	(A)Shoot fresh weight	0.350	<.001	0.021		0.105	0.294	0.174
	(A)Root fresh weight	0.009	0.004	0.037		0.003	0.734	0.901
	(B) Shoot dry weight	0.545	<.001	0.002		0.118	0.032	0.915
	(B) Root dry weight	<.001	0.200	0.544		0.171	0.077	0.695
	(C)Root:Shoot ratio	0.102	<.001	0.074		0.691	0.935	0.574

Table 3. Pearson's correlation matrix for maize plant physiology attributes investigated during this chapter in plants three weeks after germination. Pearson correlation coefficients are plotted with highlighted coloured shading representing significance at 0.05 level (two-tailed) = and at 0.001 level (two-tailed) = .

	Number of stomatal files	Canopy area	Canopy height	G_{smax} water	Intrinsic WUE (at 1500ppfd)	Leaf temperature	Leaf weight ratio (thickness)	ΦPSII (at 1500ppfd)	Root dry weight	Total root length	Root:Shoot ratio	Root surface area	Root volume	Rooting depth	leaf relative water content RWC	Shoot dry weight	G_{smax} CO_2	Specific leaf area (SLA)	Specific leaf mass (SLM)	Percentage of open stomata	Stomatal conductance (at 1500ppfd)	Stomatal density	Stomatal size	Assimilation	Transpiration
Number of stomatal files	1																								
Canopy area	-0.1592	1																							
Canopy height	-0.0088	0.9135	1																						
G_{smax} water	0.4592	-0.0843	0.0962	1																					
Intrinsic WUE (at 1500ppfd)	0.4063	0.3971	0.3839	0.2131	1																				
Leaf temperature	0.035	-0.1342	-0.3723	-0.0104	0.2402	1																			
Leaf weight ratio (thickness)	-0.176	0.7489	0.7665	-0.0198	0.3644	0.0964	1																		
ΦPSII (at 1500ppfd)	-0.3102	0.1921	0.2406	0.2005	-0.2198	-0.4891	-0.1992	1																	
Root dry weight	-0.5338	0.2689	0.2748	-0.1305	-0.2934	-0.5573	0.179	0.1496	1																
Total root length	-0.0212	0.7871	0.8886	-0.0087	0.1848	-0.6314	0.5639	0.2517	0.5332	1															
Root:Shoot ratio	-0.2797	-0.7436	-0.7107	0.0297	-0.2966	-0.0422	-0.5458	-0.0833	0.3126	-0.5025	1														
Root surface area	-0.168	0.7786	0.8479	-0.0312	0.0896	-0.6143	0.5399	0.2285	0.6326	0.9303	-0.4228	1													
Root volume	-0.5031	-0.5628	-0.6722	-0.3257	-0.6637	0.1586	-0.4338	-0.068	0.2602	-0.4716	0.6924	-0.3618	1												
Rooting depth	-0.2666	0.9061	0.8651	-0.2907	0.2434	-0.2824	0.6718	0.2351	0.3307	0.7451	-0.6698	0.7636	-0.4517	1											
leaf relative water content RWC	-0.1214	-0.703	-0.7557	-0.277	-0.7463	-0.1042	-0.8079	0.0408	0.0748	-0.5038	0.5234	-0.4535	0.6271	-0.5525	1										
Shoot dry weight	0.0313	0.9601	0.8956	-0.0009	0.4817	-0.1387	0.6568	0.1219	0.1701	0.7507	-0.78	0.7781	-0.6617	0.8628	-0.6979	1									
G_{smax} CO_2	0.4592	-0.0843	0.0962	1	0.2131	-0.0104	-0.0198	0.2005	-0.1305	-0.0087	0.0297	-0.0312	-0.3257	-0.2907	-0.277	-0.0009	1								
Specific leaf area (SLA)	0.0849	-0.7657	-0.6687	0.0765	-0.5749	-0.3042	-0.8877	0.3254	-0.0688	-0.4011	0.5638	-0.4289	0.489	-0.6949	0.7966	-0.7425	0.0765	1							
Specific leaf mass (SLM)	0.046	0.723	0.7119	0.1279	0.7026	0.2447	0.9074	-0.2615	-0.0253	0.4405	-0.5434	0.4079	-0.6187	0.5824	-0.9292	0.7008	0.1279	-0.9393	1						
Percentage of open stomata	-0.6332	-0.4698	-0.5538	-0.3915	-0.8046	-0.1215	-0.4705	0.205	0.4186	-0.3147	0.6151	-0.2589	0.8098	-0.3106	0.7966	-0.612	-0.3915	0.5825	-0.72	1					
Stomatal conductance (at 1500ppfd)	-0.7354	-0.0046	-0.0421	-0.2011	-0.7043	-0.2844	-0.1538	0.6972	0.2953	0.0048	0.1323	0.1324	0.4736	0.1201	0.3321	-0.1353	-0.2011	0.3461	-0.4191	0.63	1				
Stomatal density	0.6232	-0.0586	0.1211	0.8927	0.3802	0.2021	0.1259	-0.0247	-0.4207	-0.066	-0.1757	-0.1807	-0.509	-0.2872	-0.3811	0.0224	0.8927	-0.0713	0.3017	-0.5561	-0.4409	1			
Stomatal size	-0.7164	0.0457	-0.057	-0.3378	-0.45	-0.3561	-0.1407	0.3383	0.6851	0.1065	0.4019	0.3313	0.574	0.2132	0.2603	-0.0262	-0.3378	0.1342	-0.3028	0.5459	0.6605	-0.7135	1		
Assimilation	-0.4928	0.2146	0.2419	0.0938	-0.4327	-0.512	-0.098	0.9087	0.2856	0.2742	-0.0745	0.3452	0.0329	0.2448	0.1046	0.1304	0.0938	0.286	-0.2591	0.3114	0.8552	-0.1611	0.4901	1	
Transpiration	-0.7194	-0.0035	-0.0387	-0.1687	-0.6935	-0.3005	-0.1895	0.7329	0.3117	0.0261	0.1472	0.1392	0.4763	0.1073	0.345	-0.1331	-0.1687	0.3832	-0.4472	0.6452	0.9935	-0.4159	0.6438	0.8637	1

Table 4. Pearson's correlation matrix for wheat plant physiology attributes investigated during this chapter in plants three weeks after germination. Pearson correlation coefficients are plotted with highlighted coloured shading representing significance at 0.05 level (two-tailed) = and at 0.001 level (two-tailed) = .

	Number of stomatal files	Canopy area	Canopy height	G_{smax} water	Intrinsic WUE (at 1500ppfd)	Leaf temperature	Leaf weight ratio (thickness)	ΦPSII (at 1500ppfd)	Root dry weight	Total root length	Root:Shoot ratio	Root surface area	Root volume	Rooting depth	leaf relative water content RWC	Shoot dry weight	G_{smax} CO_2	Specific leaf area (SLA)	Specific leaf mass (SLM)	Percentage of open stomata	Stomatal conductance (at 1500ppfd)	Stomatal density	Stomatal size	Assimilation	Transpiration
Number of stomatal files	1																								
Canopy area	-0.4216	1																							
Canopy height	-0.3737	0.436	1																						
G_{smax} water	0.6394	-0.4828	-0.5906	1																					
Intrinsic WUE (at 1500ppfd)	-0.1438	-0.3167	-0.044	0.3411	1																				
Leaf temperature	-0.3464	0.2302	-0.1226	-0.4097	0.0815	1																			
Leaf weight ratio (thickness)	-0.0036	0.14	0.5587	-0.1334	-0.1654	-0.0755	1																		
ΦPSII (at 1500ppfd)	-0.5226	0.6732	0.6274	-0.4602	-0.1284	0.1713	0.6154	1																	
Root dry weight	-0.1709	0.439	0.3786	-0.1986	-0.2596	0.0086	0.3091	0.452	1																
total root length	-0.1985	0.5176	0.5302	-0.4511	-0.3952	-0.0257	0.694	0.6016	0.4452	1															
Root:Shoot ratio	0.1423	-0.0244	-0.2275	0.161	-0.1043	0.0937	-0.1233	-0.1019	0.6967	-0.0503	1														
Root surface area	-0.1366	0.4085	0.3945	-0.2858	-0.3499	-0.0718	0.6004	0.3866	0.3361	0.9244	-0.1278	1													
Root volume	-0.5266	0.6088	0.569	-0.3967	-0.2864	0.0745	0.4001	0.5235	0.532	0.4939	-0.0534	0.5729	1												
Rooting depth	0.1093	0.1228	0.7543	-0.1946	-0.0467	-0.2336	0.4762	0.4174	0.4745	0.1721	0.1375	-0.0149	0.2135	1											
leaf relative water content RWC	0.026	0.1758	0.6387	-0.1793	-0.038	0.1101	0.8082	0.6708	0.4274	0.4487	0.0315	0.2932	0.3041	0.7683	1										
Shoot dry weight	-0.2653	0.6797	0.6768	-0.4022	-0.4031	-0.0591	0.567	0.7053	0.808	0.7588	0.183	0.682	0.7744	0.5079	0.5822	1									
G_{smax} CO_2	0.6394	-0.4828	-0.5906	1	0.3411	-0.4097	-0.1334	-0.4602	-0.1986	-0.4511	0.161	-0.2858	-0.3967	-0.1946	-0.1793	-0.4022	1								
Specific leaf area (SLA)	0.4281	-0.2875	-0.2798	0.0087	-0.3384	0.0679	0.0958	-0.1974	-0.1321	0.3181	0.007	0.3683	-0.3531	-0.21	0.0721	-0.0483	0.0087	1							
Specific leaf mass (SLM)	-0.4051	0.3305	0.3819	-0.0357	0.2778	-0.139	0.0665	0.3219	0.1543	-0.1947	-0.0605	-0.2862	0.3728	0.3065	0.0535	0.1333	-0.0357	-0.9796	1						
Percentage of open stomata	-0.2066	0.2562	0.4601	-0.1331	-0.1687	-0.413	0.0824	0.4305	0.3056	0.12	-0.1943	0.101	0.3925	0.4558	0.2957	0.5147	-0.1331	-0.0912	0.1344	1					
Stomatal conductance (at 1500ppfd)	-0.2028	0.4073	0.4478	-0.1513	-0.3868	-0.3154	0.4625	0.6803	0.7285	0.4899	0.2202	0.3678	0.5207	0.4896	0.5096	0.806	-0.1513	-0.051	0.1493	0.7468	1				
Stomatal density	0.8178	-0.5825	-0.6771	0.9162	0.2453	-0.2597	-0.256	-0.6457	-0.3471	-0.5623	0.1657	-0.4213	-0.6112	-0.1933	-0.2214	-0.5798	0.9162	0.1778	-0.2202	-0.3121	-0.3901	1			
Stomatal size	-0.7288	0.4628	0.4093	-0.2682	-0.0222	-0.1633	0.2712	0.6555	0.503	0.3943	0.0043	0.3515	0.6751	0.0877	0.1449	0.6055	-0.2682	-0.4197	0.4606	0.5563	0.7191	-0.6224	1		
Assimilation	-0.5376	0.4306	0.5775	-0.3363	-0.1061	-0.0739	0.6326	0.8477	0.5971	0.587	0.0029	0.4388	0.601	0.383	0.5609	0.7447	-0.3363	-0.2225	0.3313	0.5396	0.8399	-0.6235	0.8587	1	
Transpiration	-0.2535	0.4164	0.4717	-0.1807	-0.3675	-0.2978	0.4749	0.7086	0.7127	0.4969	0.187	0.3739	0.5428	0.4841	0.517	0.8066	-0.1807	-0.0713	0.1706	0.7543	0.9981	-0.4279	0.7494	0.8644	1

5.5 DISCUSSION

Overall, in maize, high humidity resulted in a greater proportion of stomata remaining open, regardless of soil moisture condition, and greater root growth (increased volume, surface area, total root length, and dry weight), suggesting high humidity is promoting a more productive plant that is capable of harnessing more soil resources. However, when soil moisture was low, high humidity led to reduced ΦPSII, net assimilation, and WUE, which could have a more profound impact on plant productivity when exposed to these conditions over a longer period.

Wheat, on the other hand, showed far greater sensitivity to soil moisture conditions, whereby high soil moisture led to increased shoot biomass production. Though humidity effects were soil moisture dependent. When soil moisture was high, high humidity led to the greatest proportion of open stomata, boasting the highest stomatal conductance, and performing at almost 50% of the g_{smax}. Whereas when soil moisture was low, high humidity resulted in stomatal morphology comparable to those grown in high soil moisture conditions, with larger stomata, more sparsely arranged and subsequent lower calculated g_{smax} values.

Hypotheses revisited

1. Root system architecture will be unaffected by humidity

We reject this hypothesis for both maize and wheat. Whilst maize exhibits humidity responses with regards to root volume, surface area, and total root lengths, wheat shows a humidity effect with regards to the root surface area to volume ratio.

2. Low soil moisture will result in increased rooting depths.

We reject this hypothesis for both maize and wheat, as both exhibit shallower rooting during low soil moisture conditions.

3. Maize stomata will remain open in high humidity regardless of soil moisture content.

We accept this hypothesis for maize, echoing results from Chapter 2

4. Wheat stomata will remain closed under low soil moisture, regardless of humidity.

We accept this hypothesis for wheat, echoing results from Chapter 2.

5. High humidity will increase ΦPSII in maize

Though accepted in Chapter 2, we, however, reject this hypothesis in this chapter, as reduced ΦPSII is observed in high humidity grown maize when measured at PPFD 1500μmol m^{-2} s^{-1}.

6. Intrinsic water use efficiency (WUE) will be highest in plants grown under low soil moisture conditions.

We accept this hypothesis for wheat and reject for maize. Maize WUE is significantly higher in low humidity and not affected by soil moisture.

5.5.1 Maize

5.5.1.1 Root System Architecture

A deeper rooting strategy is often adopted by plants growing in low soil moisture environments and is therefore considered a key drought avoidance strategy (Henry *et al.*, 2012; Lynch, 2013). As such it is commonly reported in the literature (Hund, Ruta and Liedgens, 2009; Lynch, 2013). However, in this chapter maize roots were shallower and had a total reduced root length in low soil moisture conditions (Figure 2), contradicting the common literature but supporting findings from Chapter 2 and a study on maize by Eghball and Maranville (1993). With rooting depth findings here, echoing those in Chapter 2, the maize plants may be experiencing severe resource depletion during low soil moisture conditions (as discussed in Chapter 2), which are consequently causing the plants to adopt a severely hindered root growth strategy, through shallower, less dense root systems (Rich and Watt, 2013). Other possibilities for reduced root lengths are nutrient deficient foraging for immobile topsoil nutrients, potential response to top-down watering technique, and possible effects of soil compaction of root growth and foraging strategy. All possibilities are discussed in Chapter 2.

There could also be an aboveground influence to belowground organ growth, as a positive linear relationship was found between rooting depth and canopy area

(Figure 20), echoing findings from previous studies (Schenk and Jackson, 2002; Bevan, Los and North, 2014). It is possible that although rates of transpiration did not differ between treatments in maize (Figure 6H) the larger canopy areas, could have resulted in higher evapotranspirational demands (Schenk and Jackson, 2002), thus increasing the need for soil-derived water. This pressure from the shoots leads to greater rooting depths to explore previously untapped soil moisture reserves (Bauerle *et al.*, 2008; Nibau, Gibbs and Coates, 2008; Henry *et al.*, 2012) to support the productive shoot growth. Though such growth is not without its costs as it has been reported that that the metabolic costs of soil exploration can exceed half of daily photosynthesis, (Lambers, Atkin and Millenaar, 2002b), with larger root systems requiring larger photosynthetic input. Therefore, with increased canopy area there is a greater amount of photosynthetic area to support the extensive root growth, hence the positive relationship between the two (Figure 20),

Due to the non-destructive and detailed investigative nature of using μCT to analyse root system architectures, this chapter observed several novel findings that would be more difficult to detect with the more destructive root washing sampling. When soil moisture was low, high humidity resulted in root system more similar to those grown under high soil moisture conditions (Figure 2). The high humidity conditions resulted in increased root volume, root surface area, total root length as well as increased overall root dry weight when soil moisture was low (Figure 2). Enhanced root growth is usually a sign of increased demand for water (Hepworth, *et al*., 2016). Though maize exhibited no increase in stomatal conductance in high humidity, nor any significant increase in

photosynthetic parameters which would have been indicative of a more productive plant.

Perhaps the low VPD (high humidity) conditions, altered the maize plants 'stress perception' of the dry soil and subsequent signalling (ABA and /or hydraulic), resulting in root growth similar to plants grown in high soil moisture conditions. Signalling within the plant in response to stressful conditions is a widely contested area in plant physiology studies. The signal itself has been debated over the years, with the most recent consensus being that plants require both hydraulic and ABA-induced signals. For a comprehensive review on signalling see Buckley (2019). In this chapter, perhaps, the increased root growth is caused by signals from the unstressed aerial organs in high humidity, low VPD conditions, to the stressed roots in dry soil moisture, encouraging enhanced root growth to maximise the soil-to-root interface, a trait associated with greater drought tolerance (Hurd, 1974). In Chapter 3 we saw high maize shoot ABA concentrations in low soil moisture, with high humidity resulting in lower root ABA concentrations when compared to LHLS conditions. Perhaps the [ABA] is still high enough to induce increased root growth in HHLS but not so high that it inhibits growth causing overall reduced root length as seen in LHLS (Figure 2). This would not be the first observation of an aboveground environmental factor affecting belowground root growth, as Berntson and Woodward (1992) found elevated CO_2 concentrations (700 μmol mol^{-1}) increased root lengths, foraging capacity and root branching in *Senecio vulgaris* during low soil moisture conditions when compared to ambient CO_2 growth treatments. The authors suggest the ability of aboveground conditions

(high CO_2) concentrations could help mitigate the effects of belowground stresses (low soil moisture).

Another concept, that has been gaining traction in recent years is that of foliar water uptake (FWU) and hydraulic redistribution of water to sites in need of rehydration (Schreel and Steppe, 2020). It is possible that during this chapter, when soil moisture is low but humidity is high, FWU could perhaps occur. It is now understood that FWU only requires leaf water potential to be slightly higher than that of the root, with the movement of water helped by gravitational water potential rather than hindered (Schreel *et al.*, 2019).This process could have redistributed potential foliar acquired water from the humid conditions, to the roots, resulting in the higher fresh and dry root weights in HHLS when compared to LHLS treatment (Figure 22). Though it is perhaps more likely the high humidity conditions could be mitigating the negative effects of drought (Eller, Lima and Oliveira, 2013) in maize by enabling the continuation of turgor-driven growth, in dry soil, through the maintenance of plant water potentials due to reduced transpirational demand and stomatal control.

Though root system architecture changed in response to both humidity and soil moisture conditions, the root:shoot dry weight ratio remained strongly influenced by soil moisture (Figure 22). During dry soil conditions, root growth is favoured over shoot growth (Sharp *et al.*, 2004), as such an increased root:shoot ratio is usually indicative of increased root growth, to increase foraging capacity and resource (water and nutrient) acquisition from the soil (Fitter and Stickland, 1991). It is generally accepted amongst the literature that an increase in the

root:shoot ratio is advantageous to plants experiencing periods of drought whereby soil resources are limited (Fitter and Stickland, 1991; Grzesiak *et al.*, 2002; Sharp *et al.*, 2004). Therefore, it is possible that the increased root:shoot ratios of maize (Figure 22) under low soil moisture conditions (regardless of humidity) and highly efficient root system architecture, tailored to the prevailing environmental conditions (both above and below the ground) helps to ensure the plant's water needs are met (Hund, Ruta and Liedgens, 2009), thus reducing the risk of hydraulic failure without significantly lowering net carbon gain when soil moisture is low, helping to maintain maize's drought-resistant status.

5.5.1.2 Stomatal Morphology

The effects of humidity on maize stomatal morphology were only present when soil moisture was high. During well-watered conditions, high humidity led to larger stomata with a lower density and reduced number of files when compared to LHHS treatment (Figure 11). The inverse relationship between stomatal size and density is maintained (Bertolino *et al*., 2019; Franks & Beerling, 2009), echoing findings from Chapter 2. Though, in Chapter 2 the reduced stomatal density was suggested to have been potentially caused by the significantly larger leaves (in the HHHS treatment) resulting in stomata spread across a greater surface area by epidermal cell expansion (Nejad and Van Meeteren, 2005). However, in this chapter the HHHS treatment did not boast the largest leaves therefore the reduced density cannot be attributed to epidermal cell expansion. The larger stomata and reduced density could be a result of increased leaf turgor in the HHHS conditions, resulting in the possible 'swelling' of stomata and cells thereby 'pushing' cells further apart, causing potentially reduced stomatal density. As such the larger, more sparsely arranged stomata are considered to exhibit

reduced gas conductance due to the larger diffusional distances and therefore associated with lower maximum theoretical stomatal conductance (g_{smax}) (Lammertsma *et al.*, 2011) this is reflected in Figure 14 whereby the g_{smax} was significantly lower in the HHHS treatment than the LHHS treatment.

With regards to the stomatal aperture (Figure 13), maize appeared more responsive to humidity conditions, with only considerable closure occurring in the LHLS treatment (comparable to results from Chapter 2). Interestingly, as seen in Chapter 2, when soil moisture was low, high humidity resulted in a greater proportion of stomata remaining open despite low soil moisture conditions (Figure 13). This further supports the idea presented in Chapter 2 that maize stomata could be directly responding to changes in VPD (Lange *et al.*, 1971; Holbrook *et al.*, 2002), regardless of soil moisture conditions.

5.5.1.3 Gas Exchange and Productivity

Maize stomatal conductance and photosynthetic capacity appear more responsive to changes in humidity than soil moisture. This could likely be due the drought tolerant, C4 nature of maize. Having adapted to a range of environments from the very wet to the very dry, sea-level to highlands (Ureta *et al.*, 2012) since its domestication between 6600 and 4700 years ago in Mexico (Piperno and Flannery, 2001). Maize can maintain high levels of photosynthesis and transpiration, despite the risks of water depletion and whole plant hydraulic failure, before the end of the crop cycle (Hund, Ruta and Liedgens, 2009) it is therefore well-equipped to cope with changing soil moisture conditions, and less likely to respond as strongly as the more soil moisture-sensitive wheat (C3), which is far less drought tolerant.

Though maize appears relatively resistant to changes in soil moisture content it does, however, respond to humidity. During this chapter, high humidity resulted in significantly lower net carbon assimilation and intrinsic water use efficiency (WUE), ΦPSII and Fv'/Fm' values (Figure 6 and Figure 9) regardless of soil moisture conditions. Plants tend to increase their water use efficiency when faced with water deficit conditions (Waraich and Ahmad, 2010) since maize WUE decreased during high humidity, regardless of soil moisture could be an indication that humidity is ameliorating some of the drought stress perceived by maize. It is possible, that the lower VPD conditions brought about by high humidity are reducing the transpirational demand on maize (though transpiration rates remained constant across treatments Figure 6H), putting less pressure on the water supply from the roots, leading to less drought-stressed plants and reducing the risk of hydraulic failure. The reduced perception of drought stress when humidity is high could therefore be leading to the reduction in WUE observed in this chapter in maize. Reduced WUE is generally associated with lower stomatal conductance (Lawson and Blatt, 2014), though this chapter saw no significant differences in stomatal conductance. The reduction in WUE could also be attributed to a majority of stomata remaining open in high humidity conditions, and under high soil, moisture stomata were larger, therefore, possessing a greater pore area for potential water loss.

Furthermore, net assimilation (Figure 6) and ΦPSII (Figure 9) were also reduced in high humidity conditions, which could also contribute to the reduced WUE, considering WUE is typically defined as the amount of carbon fixed by

photosynthesis per unit of water expired (Lawson and Blatt, 2014). In terms of carbon available to plant it is possible that during high humidity conditions, the diffusivity of CO_2 through the humid air was reduced, as gas diffusion coefficients are considered to decrease when relative humidity increases (Benavente and Pla, 2018). This alteration in the diffusivity of CO_2 could perhaps have led to localised reductions in CO_2 around the leaf, caused by CO_2 uptake from the plant and reduced diffusion of CO_2 through the surrounding air to replace the localised uptake of the compound. If less CO_2 is available for the plant, then net assimilation, photosynthetic processes, and water use efficiency will all be reduced, something that was observed during this chapter in maize during high humidity conditions. However, due to the air mixing capabilities of the growth chambers (Conviron A2000) used in this chapter, it is unlikely that this occurred during this chapter. It could have perhaps played a role in Chapter 2 whereby limited air mixing occurred in the glasshouse growth chamber.

The reduced net assimilation (Figure 6) and the lower overall net assimilation at a given stomatal conductance (Figure 8) under high humidity conditions, could explain the lower shoot dry weights observed in maize when soil moisture was also high (Figure 22). Though in low soil moisture conditions, despite the reduced net assimilation in high humidity, shoot biomass was slightly higher when compared with the LHLS treatment. These results suggest that high humidity could hinder biomass production in maize when soil moisture is high but help biomass production when soil moisture is low. However, the possible hindrance of high humidity to photosynthesis and net assimilation may not be as prevalent in natural field conditions, since the very high humidity conditions witnessed in this

chapter (~76-90% RH), would often be accompanied by extensive cloud cover in the natural environment, as relative humidity increases with increasing cloud cover (Walcek, 1994; Groisman, Bradley and Sun, 2000; Betts *et al.*, 2014) during these conditions the incidence radiation will be generally lower and could, therefore, render the expected negative impact of humidity on photosynthesis negligible (Eller, Lima and Oliveira, 2013). A study on the effects of high humidity on maize photosynthesis under a range of incidence radiation levels is needed to aid predictions of responses to field conditions.

5.5.2 Wheat

5.5.2.1 Root Architecture

In wheat, high humidity did not affect root volume, surface area or total root length, it did however significantly reduce the root surface area to volume ratio (Figure 4) -regardless of soil moisture content- suggesting high humidity favours increased root volumes over relation to the surface area. This was an unexpected result particularly for the low soil moisture treatment, as drought conditions, and low water potentials cause roots to become thinner, to reduce metabolic costs and pool resources (Sharp *et al.*, 2004; Hund, Ruta and Liedgens, 2009; Lynch, 2013), perhaps the high humidity is causing the wheat roots to adopt a different strategy when soil moisture is low. Plants can reduce resistance to water movement from the soil to the root by increasing the xylem diameters (Wasson *et al.*, 2012) and a correlation of root thickness and xylem size has been observed in rice (Yambao, Ingram and Real, 1992). Perhaps this is what we are seeing in this chapter, where high humidity is causing wheat roots to increase thickness to accommodate larger xylem vessels, to facilitate increased water uptake and movement from the roots.

Moreover, the reduced root surface area to volume ratio of the wheat root could not only be driven by an increase in root volume but also a decrease in root surface area, through reductions in lateral root and root hair formation during low soil moisture conditions. Although lateral roots and root hairs increase the total surface area of the RSA and aid soil exploration in resource deficit conditions (Nibau, Gibbs and Coates, 2008), they are metabolically expensive (Hepworth, *et al*., 2016), therefore decreasing the root surface area through reductions in lateral root and root hair formation could help reduce the metabolic costs associated with soil exploration in water deficit environments. Though changes to specific root type we not documented, we cannot say whether high humidity caused a reduction in lateral root formation.

Overall wheat roots were not drastically affected by the treatment conditions, with no effect on dry weight production (Figure 23) and only a humidity effect on the root volume to surface area ratio (Figure 4). These findings are not conducive to those in Chapter 2 whereby wheat roots appeared very responsive to both soil moisture and humidity conditions. With reduced dry weight when soil moisture was low and reduced dry weight in high soil moisture conditions when humidity was high. The major difference between these two experiments was that the Chapter 2 experiment was carried out in a glasshouse and the experiment in this current chapter was carried out in a controlled growth chamber. Perhaps the changes to root biomass observed in Chapter 2 were affected by a greater number of environmental factors that could not be as well controlled in the glasshouse such as temperature and light intensity. The temperatures reached in the glasshouse experiments were more well suited to maize growth rather than wheat,

which could mean that the responses observed in wheat during Chapter 2 could have been driven by high temperature stress rather than the treatment conditions themselves.

5.5.2.2 Stomatal Morphology

The only large significant changes to wheat stomatal morphology occur when both humidity and soil moisture are low. Smaller stomata, more densely arranged, with a higher number of files are typical of the LHLS treatment, echoing findings from Chapter 2. These results could be a result of reduced leaf turgor (as discussed in Chapter 2), driven by low soil moisture conditions and subsequently reduced soil water potential (Rodriguez-Dominguez *et al.*, 2016) and leaf dehydration (Kim *et al.*, 2018). Such a reduction in leaf turgor would lead to smaller cells, of which the 'shrinkage' would draw cells closer together, increasing the cell density. Since stomatal density is inevitably affected by the cell density on the leaf surface (Wang, Chen and Xiang, 2007), any increase in leaf cell density will result in a subsequent increase in stomatal density. The LHLS treatment did also boast the shortest canopy (Figure 21), though there were no significant changes to specific leaf area compared to other treatments. Smaller leaves could explain the increased stomatal densities and number of files through reduced epidermal cell expansion (Nejad and Van Meeteren, 2005). However, no significant reductions in specific leaf area were recorded, suggesting that the leaf size did not differ substantially between the treatments.

Smaller stomata, which are more densely arranged, are considered to possess the ability to respond faster to fluctuating light conditions and changes in atmospheric humidity (VPD) (El-Sharkawy and Cock, 1986; Franks and Farquhar, 2006;

Drake, Froend and Franks, 2013), more rapidly than larger stomata (Hetherington and Woodward, 2003). Furthermore, smaller stomata packed at higher densities can exhibit greater gas conductance due to the shorter diffusion distances (Raven, 2014). The rapidity of stomatal response and reduced diffusion distances of smaller, densely arrange stomata are therefore associated with aiding WUE (Drake, Froend and Franks, 2013) and increasing the maximum theoretical stomatal conductance (g_{smax}) of a plant (Hetherington and Woodward, 2003; Franks and Beerling, 2009; Lammertsma *et al.*, 2011). As such, high WUE and the highest g_{smax} values were recorded in the LHLS treatment which consequently possessed the smallest, most densely arranged stomata.

Interestingly, when comparing wheat operational g_s to theoretical g_{smax} the wheat growing in the HHHS treatment was performing significantly closer to the theoretical g_{smax} (approximately 45-50%) when compared to the other treatments <20%) (Figure 17). A contrast to maize whereby treatments did not affect the consistently low stomatal conductance performance, of their theoretical g_{smax}. (<10%) (Figure 16). A difference of performance was somewhat expected as g_s differs from g_{smax} depending on species and the growth conditions (Conesa *et al.*, 2020). Stomata respond dynamically to changes in environmental conditions and therefore the theoretical g_{smax} is rarely observed in field conditions (Hemsley and Poole, 2004; Dow, Bergmann and Berry, 2014). A study by McElwain, Yiotis and Lawson (2016) highlighted the variations between different species with regards to how close to the theoretical maximum (g_{smax}) they are performing, such variety could not be clearly explained by biogeography, habit, ecology or phylogeny. The study suggested that species with higher net photosynthetic rates can enable

higher g_s closer to the theoretical maximum (McElwain, Yiotis and Lawson, 2016). However, during this chapter, the wheat in the HHHS treatment did not exhibit significantly higher ΦPSII (Figure 10) or net assimilation (Figure 7), though it did boast the highest rates of stomatal conductance (g_s) (Figure 7). It is suggested that there is a tendency for increased g_s within species to occur through reductions in stomatal size and increases in density (Hetherington and Woodward, 2003; Franks, Drake and Beerling, 2009). However, the higher g_s HHHS plants exhibited larger, lower density stomata. Though stomatal morphology (larger/less dense), did not necessarily promote higher g_s, perhaps the environmental conditions were sufficient to drive higher g_s, performing far closer to the g_{smax} than any other treatment. The HHHS provided ample soil water and low VPD from the high humidity. A majority of stomata were open, and with little reason to close, they exhibited the highest rates of stomatal conductance and subsequent transpiration, and the lowest WUE in HHHS compared to all other treatments.

With regards to stomatal morphology, during low soil moisture conditions, when humidity was high, the stomatal morphology (size, density, and the number of files) appeared more similar to the plants growing in high soil moisture conditions (Figure 12). These results suggest that the stomatal morphology is responding directly to the high humidity (low VPD) conditions, and echo findings from Chapter 2, whereby the results are discussed in more detail. Other findings, comparable to those in Chapter 2 are that both low humidity and low soil moisture led to stomatal closure in wheat and only a large proportion of open stomata was witnessed when both humidity and soil moisture were high (HHHS) (Figure 13). These results, therefore, suggest that when soil moisture is high, low humidity

(high VPD) is enough to induce stomatal closure in wheat despite ample soil moisture. Whereas when soil moisture is low, high humidity (low VPD) is not enough to warrant the opening of stomata (as seen in maize), low soil moisture appears the dominant driver of stomatal aperture in wheat.

5.5.2.3 Gas Exchange and Productivity

Plant responses to humidity and soil moisture, in terms of stomatal conductance, photosynthetic capacity, and biomass production vary considerably between species. In this chapter, wheat appeared more sensitive to changes in soil moisture, whereas maize was more sensitive to changes in humidity.

During low soil moisture conditions, wheat plants showed significantly lower stomatal conductance, net assimilation, ΦPSII and Fv'/Fm' values (Figure 7 and Figure 10) and an increase in water use efficiency (Figure 7). Such responses are typical of drought-stressed plants, and for wheat, humidity did not influence the plant's response to such conditions. In the low soil moisture conditions, wheat plants closed a majority of their stomata (Figure 13) thus reducing the availability of CO_2 (Waraich and Ahmad, 2010) for critical photosynthetic enzymes such as Rubisco, leading to net reductions in net assimilation and reduced stomatal conductance (Figure 7) to mitigate water loss. As such, we see an increase in the WUE of wheat plants grown in low soil moisture treatment conditions (Figure 7).

Changing WUE behaviours in response to environmental stimuli are deemed an important parameter for crop simulation models (Steduto and Albrizio, 2005) and a key component of drought-resistant strategies. The increased WUE in wheat during low soil moisture conditions is a response that is common throughout drought tolerance literature (Grzesiak *et al.*, 2002; Hund, Ruta and Liedgens,

2009; Waraich and Ahmad, 2010), supporting findings in this study that suggest wheat is adopting a more conservative, water-saving strategy upon which humidity has little or no effect. However, such an effective strategy is not without its costs as reduced stomatal conductance and photosynthetic capacity not only reduce water lost but also carbon gained. Net carbon assimilation rates are a considerable factor for productivity (Waraich and Ahmad, 2010) and are controlled by stomatal conductance, specific metabolic processes and photosynthetic capacity (Waraich and Ahmad, 2010), as such, wheat experienced a reduction in shoot dry weights under low soil moisture conditions (Figure 23), most likely due to the reduced carbon assimilation. Unlike in maize, high humidity conditions have no considerable impact (positive or negative) on wheat in terms of stomatal conductance, photosynthetic capacity, or biomass production, though it could be impacting the efficiency of carbon gain. When looking at the relationship between wheat stomatal conductance and carbon assimilation (Figure 8), it is clear that the high humidity high soil moisture (HHHS) treatment experiences the highest stomatal conductance values (most likely driven by the vast majority of stomata remaining open), but net carbon assimilation rates fail to increase proportionally with stomatal conductance in wheat, the HHHS treatment displays the weakest relationship between assimilation and stomatal conductance. These findings, along with the low WUE and high rates of transpiration in HHHS suggest that growing wheat in both high soil moisture and high humidity results in a more water inefficient plant that is not effectively utilising the ample water source.

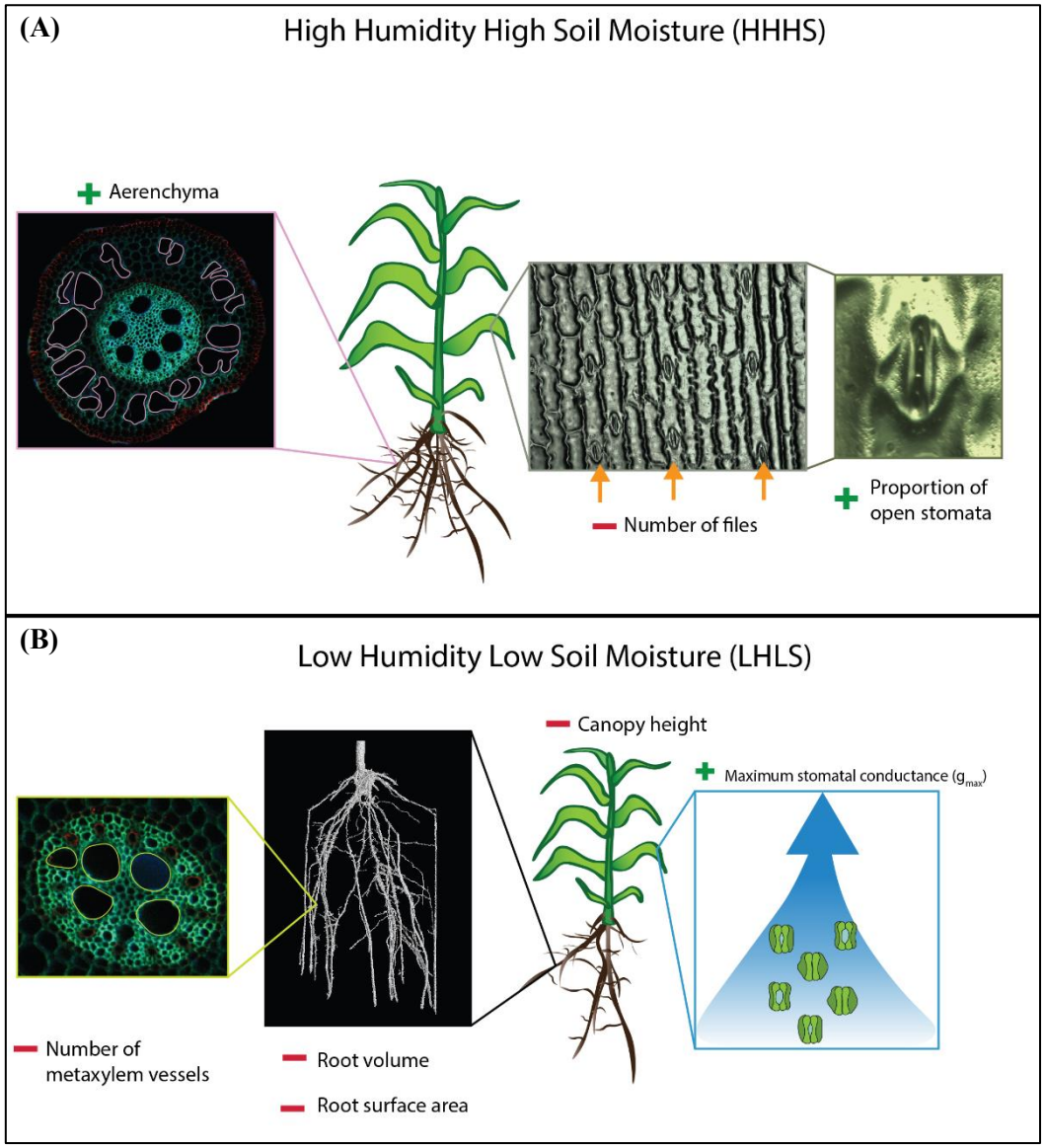
5.6 CONCLUSION

Both maize and wheat exhibited changes to whole plant physiology in response to differing humidity and soil moisture treatments. Whilst maize appeared to show greater sensitivity to changes in humidity, wheat exhibited greater sensitivity to changes in soil moisture. This chapter has therefore highlighted the importance of studying whole-plant physiological responses to changing environmental condition both above and below the surface. Aboveground organ responses are not confined to aerial conditions nor are belowground organs solely responding to soil conditions. The effects of humidity and soil moisture have exhibited both independently driven changes to plant physiology and co-depending effects whereby one treatment effect is reliant on the other treatment. For example, some of the high humidity responses on maize and wheat were only observable when soil moisture was low, such as increased root volume and surface area in maize and increased stomatal density and subsequent reduction in stomatal size in wheat. Such co-dependence further strengthens the notion that the effects of humidity and soil moisture on plant physiology should be investigated together, as one may influence the plant 'sensitivity' to the other, both above and below the ground.

In addition, particular physiological responses to high humidity in low soil moisture conditions such as increased root growth (surface area, volume, root length) for maize, suggests that high humidity has the potential to offset some of the stresses induced by dry soil conditions, increasing soil exploration and supporting larger canopies. However, high humidity can also reduce the photosynthetic capacity, net assimilation and WUE of maize. Raising the question of how much help or a hindrance the environmental variable truly is for maize crop performance and productivity.

6 GENERAL DISCUSSION

This thesis has highlighted the importance of considering the effects of humidity and soil moisture as both dependent and interdependent influencers of plant physiology in early development stages of maize and wheat. Schematics of a whole plant perspective response, collating main results from all experimental chapters are presented for maize (Figure 24) and wheat (Figure 25). The novel treatments of high and low humidity and soil moisture have provided a unique insight into how both species perceive water stress in terms of reduced supply (low soil moisture content) or increased demand (low humidity and subsequent high VPD).



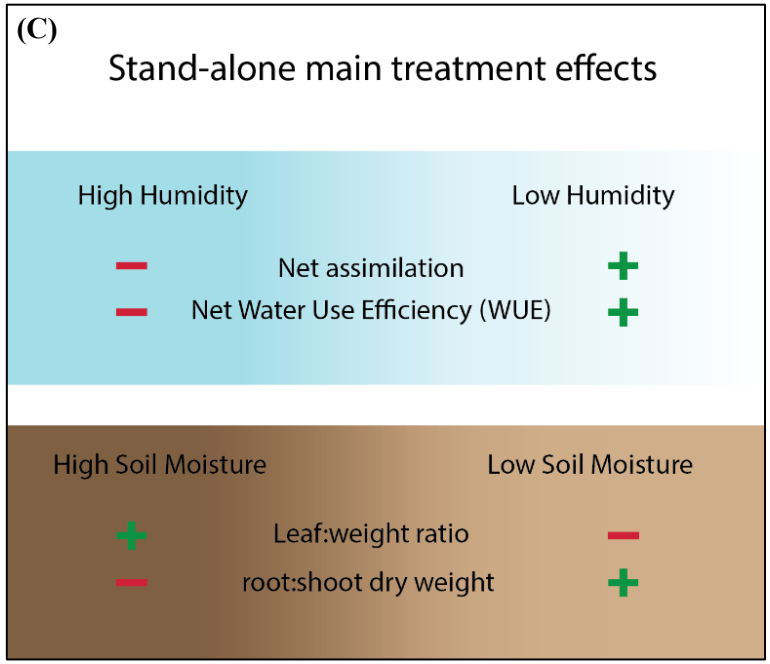
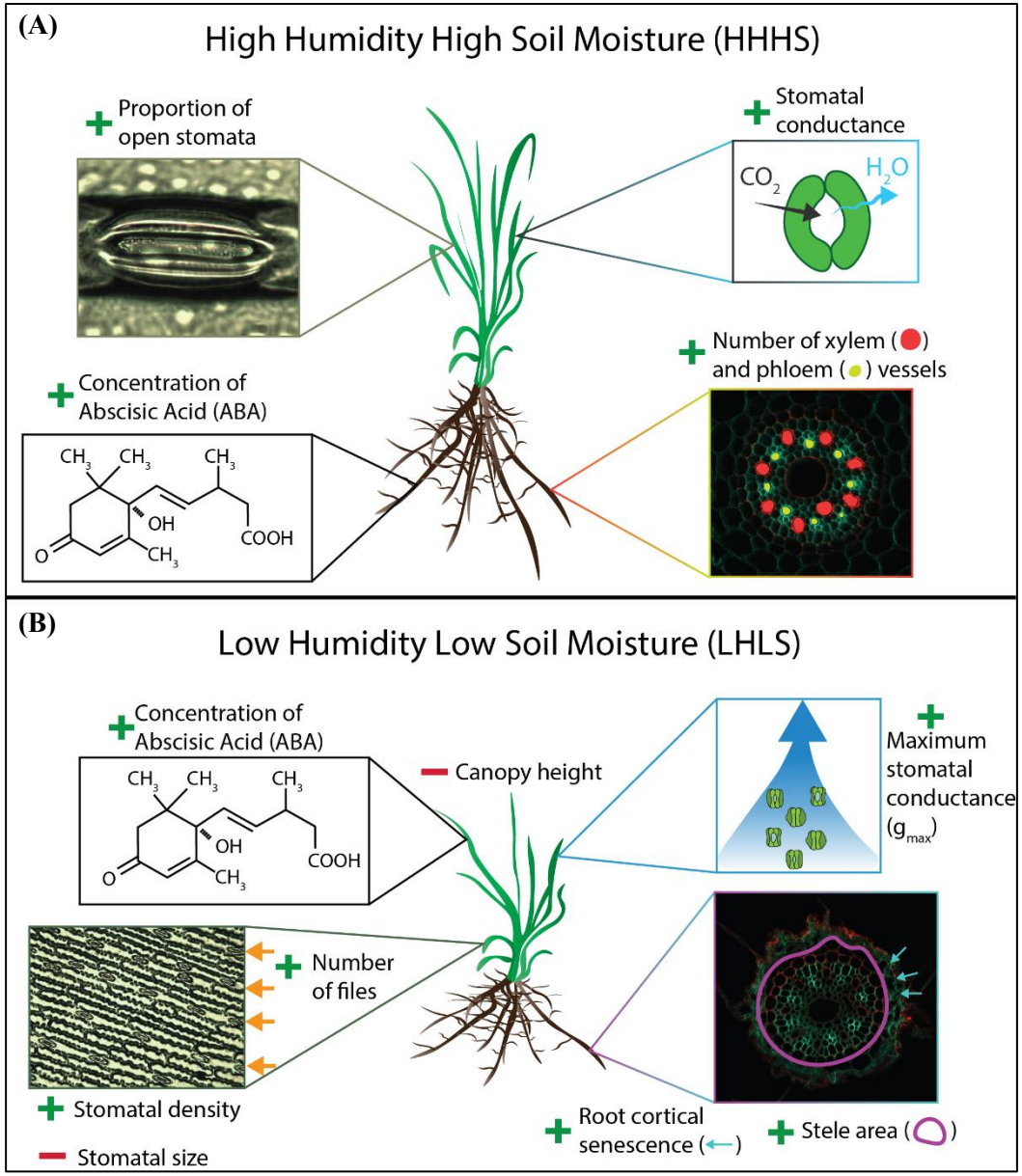


Figure 24. A schematic detailing the effects of humidity and soil moisture on maize physiology (*Zea mays*) as documented throughout the thesis. Humidity and soil moisture interdependent effects are presented in (A) HHHS and (B) LHLS, whereby both specific humidity and soil moisture conditions are required for the physiology effects detailed. Stand-alone, effects of humidity and soil moisture are listed in (C), whereby the humidity and soil moisture effects influence physiology independently, regardless of the other treatment conditions.



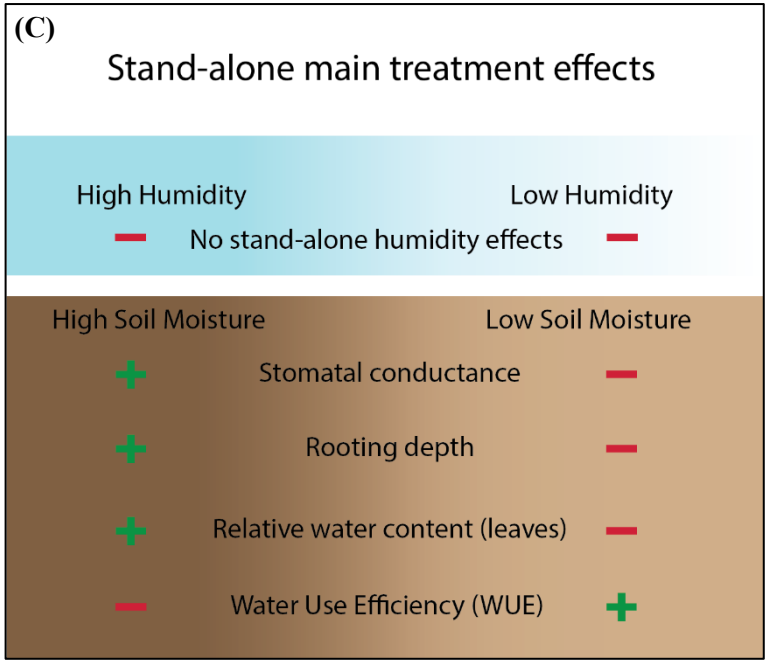


Figure 25. A schematic representing the effects of humidity and soil moisture on maize physiology (*Zea mays*) as documented throughout the thesis. Humidity and soil moisture interdependent effects are presented in (A) HHHS and (B) LHLS, whereby both specific humidity and soil moisture conditions are required for the physiology effects detailed. Stand-alone, effects of humidity and soil moisture are listed in (C), whereby the humidity and soil moisture effects influence physiology independently, regardless of the other treatment conditions.

6.1 HUMIDITY: HELP OR HINDRANCE?

The research presented in this thesis shows humidity can affect both maize and wheat physiology. The predicted rises in humidity occurring over many of the main crop-growing regions in central and eastern United States, western China (Dai, 2006), central Europe (Jones and Moberg, 2003), eastern Africa (Collier *et al*, 2008), raises the question as to whether high humidity could help or hinder future plant growth and productivity. The answer of which depends on not only the species but also the soil moisture growth conditions.

6.1.1 High Soil Moisture

6.1.1.1 Maize

In future climate scenarios whereby, humidity is predicted to rise, if irrigation is maintained resulting in high soil moisture, we could witness a rise in 'lazy' inefficient maize crops. This could be a large problem for the heavily irrigated, Corn Belt region of the American Midwest, with more volatile weather and rising humidity reported in recent years (Pryor *et al.*, 2014). This thesis showed that maize grown in HHHS had the lowest net carbon assimilation, water use efficiency (WUE), and ΦPSII (when measured at PPFD 1500µmol m^{-2} s^{-1}). Though these processes did not appear to influence the biomass production in terms of dry weight, the experimental duration may have not been long enough to see the consequences of reductions in carbon assimilation, WUE and ΦPSII. The maize plants might not just be inefficient in HHHS but also slower to respond to changing conditions. The HHHS grown maize plants boasted the largest stomata, with a majority reported as open (size reported in Chapter 2 and Chapter5). It has been suggested that larger stomata are slower to respond to changes in

environmental conditions (Hetherington and Woodward, 2003; Kübarsepp *et al.*, 2019), and are therefore favourable to plants where rapid closure is unlikely to be needed (Aliniaeifard and Van Meeteren, 2016), such as the HHHS conditions. This should not be too much of an issue to plants if the conditions remain constant, though, in our perpetual climate with more extreme weather events forecast, this is unlikely.

Furthermore, high humidity areas could also find maize stomata unable to respond to stimuli, that would usually lead to stomatal closure (Aliniaeifard and van Meeteren, 2013). High humidity (low VPD) conditions, has been observed to disrupt 'normal' stomatal functioning (Torre and Fjeld, 2001; Nejad and Van Meeteren, 2005; Arve *et al.*, 2011), namely the ability of the leaves to maintain adequate water status (Aliniaeifard and van Meeteren, 2013). This could be detrimental to maize plants, which already observe reduced net assimilation and water use efficiency when grown in high humidity. During this thesis, we have observed possible evidence for such stomatal dysfunction. Maize [ABA] concentrations in the shoots were significantly higher in low soil moisture conditions, however, only significant stomatal closure occurred when humidity was low (VPD high). The stomata in the high humidity conditions could have become insensitive and unresponsive to ABA, resulting in a lack of significant closure despite low soil moisture conditions and high shoot [ABA]. Such behaviour could be damaging to maize plants (and other humidity-sensitive plants) if the climate becomes more erratic, with periods of high and low humidity oscillating over growing seasons. A study on *Tradescantia virginiana* found that loss of stomatal functioning was achieved after just four days when plants were

transferred from moderately high to low VPD conditions (Nejad and Van Meeteren, 2005). Incidentally, when transferred back into high VPD conditions, stomatal closure recovery failed to occur (Nejad and Van Meeteren, 2005).

High humidity could therefore be detrimental to the efficiency and rapidity of maize responses to perturbations in environmental conditions. Moreover, the maize in the Midwest could be causing further detrimental effects via positive feedback mechanisms through raising local humidity levels even further by transpirational processes alone. A single acre of mature maize canopy can transpire over 15,000 litres of water each day (Suyker and Verma, 2008). Considering the U.S has more than 90 million acres dedicated to maize growth, the effects on of humidity on plant physiology across the country could therefore be very substantial.

6.1.1.2 Wheat

Wheat is less responsive to changes in humidity than maize but is also greatly influenced by soil moisture. Consequently, the HHHS conditions could be favourable for wheat, due to the greater proportion of open stomata, higher stomatal conductance and relative water content in leaves, and enhanced rooting depths found in wheat grown under high humidity and high soil moisture conditions.

Interestingly in the HHHS conditions, wheat g_s performed significantly closer to the g_{smax} (Chapter 5) (~40-60%) compared to the other treatments (~10-20%). This high performance was not necessarily expected, as due to the dynamic nature

of stomata, responding to stimuli and environmental conditions, the anatomical g_{smax} is rarely witnessed under field conditions (McElwain, Yiotis and Lawson, 2016). These results suggest that for wheat, it may be possible to raise operational stomatal conductance closer to theoretical maximum, by providing ample soil moisture and reducing the transpirational demand by lowering the VPD. It could also highlight the need to take humidity and VPD into consideration in when comparing the relationship between operational and theoretical maximum conductance, especially when such relationships are used to predict the operational conductance of extinct taxa in the fossil record. It could be possible that humidity is playing a larger part in this relationship than we thought. Though no effects were observed in maize which could be due to the age of the plant as only early developmental stages were sampled. Sampling of maize throughout the lifecycle will give us a better insight into how VPD could affect maize's operational and theoretical maximum conductance relationship.

Whist high humidity could favour wheat growth in high soil moisture conditions, one response that is prevalent in both maize and wheat which could have serious knock-on effects in terms of pathogen invasion is the predominantly open stomata. The vulnerable, stomata of both maize and wheat in HHHS conditions, are effectively 'sitting ducks' to fungal pathogen invasion (McKown *et al.*, 2014), further threatened by high humidity providing optimal growth conditions for fungal development (Piepenbring *et al.*, 2015). Therefore, unless maize and wheat can defend against fungal pathogen invasion, the large proportion of open stomata could prove problematic in HHHS conditions. As it stands, microbial disease accounts for around 16% of annual crop loss (Oerke, 2006) of which 70-

80% of such losses are caused by fungi (Moore, Robson and Trinci, 2020). An increase in global temperatures may further increase pathogen incidences which are already rising worldwide (Corredor-Moreno and Saunders, 2020).

Though high humidity could lead to less efficient maize plant and leave both maize and wheat more susceptible to disease in high soil moisture conditions. It could benefit plants grown in low soil moisture conditions.

6.1.2 Low Soil Moisture

During low soil moisture conditions, both maize and wheat exhibited beneficial responses to high humidity which suggest that the high humidity (low VPD) could be reducing the water stress perceived by the plant, leading to some changes in physiology that are more similar to their counterparts growing in high soil moisture conditions.

6.1.2.1 Maize

Though maize grown under high humidity low soil moisture conditions still exhibited reduced stomatal conductance and water use efficiency, there were several potentially beneficial high humidity effects. Interestingly, maize roots were particularly affected. During low soil moisture conditions, high humidity led to increased root dry weight, depth, volume, and surface area, albeit a more substantial root system akin to maize grown in high soil moisture conditions. It could therefore be suggested that when grown under low soil moisture conditions, an increase in humidity could benefit maize plants through increased foraging capacity of the root architecture. The increased root growth could be beneficial in reaching immobile nutrients such as phosphorous, and water reserves further from the plant. This could lead to a more productive plant, and though no significant

increase in shoot biomass or leaf area was recorded for maize in HHLS conditions compared to LHLS, a taller canopy was established, which is evidence of more resources being allocated to the aboveground organs. The plants measured were only three-weeks old, a longer-term exposure experiment whereby soil reserves become more depleted could see larger benefits to plants grown in high humidity, with more well-established root systems.

6.1.2.2 Wheat

Interestingly high humidity also caused an increase in canopy height for wheat when growing in low soil moisture conditions, though like maize, no changes to biomass were recorded. This again could be evidence that high humidity could lead to more productive plants when soil moisture is low, but the experiments conducted were not long enough to witness such changes in biomass, due to limited exposure and growth time. There is additional evidence that high humidity reduces the stress perceived by wheat when soil moisture is low, with significantly lower [ABA] recorded in the shoots compared to LHLS (Chapter 3) and the prevention of root cortical senescence (Chapter 4), both of which can be indicators of drought stress. As ABA is usually prominently located in the sites experiencing most stress, and with only significantly high concentrations recorded in the shoots of LHLS plants (not HHLS), this is further evidence that the wheat plants are perhaps not as stressed when humidity is high.

Furthermore, in wheat, more stomata (approximately 50%) were open when humidity was high, despite the low soil moisture conditions, possibly indicating some stomatal dysfunction in response to low VPD as discussed in maize, though to a lesser extent. Similarly, the stomata could just be responding directly to the

low VPD, remaining open despite low soil moisture conditions. Afterall there were significantly lower [ABA] in HHLS shoots compared to LHLS shoots suggesting less ABA is reaching the leaves to induce closure.

The lower stomatal densities and larger stomata in HHLS compared to LHLS, were more similar to wheat plants grown under high soil moisture conditions, implying that the high humidity conditions could be preventing particular changes to stomatal morphology that is associated with dry soil moisture conditions and increasing drought tolerance.

This could be a problem for wheat, should conditions vary, where high humidity is not always guaranteed. Smaller, denser stomatal arrangements are associated with drier environments, whereby greater gas conductance can be carried out over shorter diffusional distances (Raven, 2014) as well as enhanced control over the rapidity of response to environmental stimuli (El-Sharkawy and Cock, 1986; Franks and Farquhar, 2006; Drake, Froend and Franks, 2013), creating a more responsive plant with higher WUE (Drake, Froend and Franks, 2013). As such the stomatal morphology responses observed in HHLS wheat, might not be as appropriate for adverse conditions (such as low humidity), which could have detrimental impacts on overall plant growth and development.

6.2 DEGREE OF SENSITIVITY

Throughout the thesis, it became apparent that relatively, maize was more sensitive to changes in humidity, and wheat was more sensitive to soil moisture. The differing degrees of sensitivity could be a result of a plethora of factors, here we discuss the most likely possibilities.

It is possible that the difference in the degree of sensitivity was a result of their growth conditions that they are usually cultivated in. If we compare two common growth environments, the UK for wheat and the American Midwest for maize, we see that both crops are sown in the spring and harvested late summer. The two crops experience different humidity and irrigation regimes during this time, with most UK grown wheat being rainfed (El Chami *et al.*, 2015) due to the favourable humidity conditions (around 70-89% RH throughout the growing season), and maize remaining irrigated throughout whilst experiencing less rainfall and lower humidity (Iowa averages for example between 55% and 60% RH growing season average).

Maize could be more sensitive to changes in humidity, as it is not accustomed to the humidity levels reached during the high humidity treatment throughout this thesis (90-100%). Whilst soil moisture has fewer impacts on maize due to the relative drought-tolerant isohydric nature of the crop (Hugalde and Vila, 2014). The isohydric nature of maize renders the maintenance of midday leaf water potential a priority. As such, isohydric plants are considered more sensitive to changes in VPD than anisohydric plants (e.g. wheat), due to the VPD affecting the transpirational demand placed on the plant (with high VPD increasing pressures and low VPD reducing them). As such, isohydric species exhibit stricter stomatal control (showing greater sensitivity to humidity) to maintain midday leaf water potential. Whereas the general lack of significant effect of treatments on the photosynthetic parameters is likely due to the C4 nature of maize, maintaining higher efficiency than C3 under varying conditions.

Wheat, on the other hand, is found to be more sensitive to changes in soil moisture content. Perhaps the rainfed nature of the growing season influences the more 'opportunistic' nature of wheat, responding rapidly to increases and decreases in soil moisture, to adjust growth and allocation of resources accordingly. The lower sensitivity to humidity could be down to either being accustomed to the high humidities during the growing season or perhaps the response to soil moisture is so strong it overrides any other possible effects of humidity. Wheat is anisohydric, allowing for decreasing midday leaf water potentials, making it less drought tolerant than isohydric maize and therefore more sensitive to changes in soil moisture content (Hugalde and Vila, 2014).

The myriad responses of both maize and wheat above and below the ground to humidity and soil moisture highlight both the complexity of whole plant physiological responses and the importance of considering both humidity and soil moisture content. Longer-term experiments will be required to evaluate how such responses may affect processes such as grain production and reproduction. The experiments carried out within this thesis only focussed on the early developmental stages of both maize and wheat. To gain insight into whole plant responses and whether the responses are a result of adaptation or acclimation to the treatment conditions, longer term experiments involving all developmental stages need to be undertaken.

6.3 APPLICATIONS AND FUTURE RESEARCH

The results of this thesis could help provide valuable insight into the often-overlooked combined effects of humidity and soil moisture content on plant physiology. There is a need to include humidity responses when assessing the

effects of drought on crops, as humidity (and subsequent VPD) could be influencing the extent of the drought stress experienced by the plant. As such phenotype screening should also consider humidity as a driver of changes to physiology, both dependent and interdependently of soil moisture content. Phenotypes that respond strongly to high humidity, for example, could see shifts in physiology that are not conducive to the drought-tolerant goals of the screening process. For example, water use efficiency is considered a good trait for predicting drought tolerance and therefore phenotypic screening for plants with high WUE is common amongst drought-tolerant studies. However, this thesis found that high humidity led to significantly lower WUE regardless of soil moisture content in maize, as such humidity can affect the WUE and the drought tolerance of a plant, so should be considered during such screening processes.

The potential benefits of high humidity (low VPD) during drought conditions could influence future irrigation practices, whereby misting at a particular time of the day (e.g. midday) could reduce transpirational demand, maintain leaf water potentials and keep stomata open. Thereby maintaining productivity whilst minimising the threat of water loss. The results of this thesis could also influence commercial glasshouse growth methods. Although most commercial glasshouses operate under optimum conditions, carefully controlling light intensity, water availability, and temperature. Yet, despite the optimal VPD for most glasshouse grown crops to be below 1.5kPa (Shamshiri *et al.*, 2016), during the summer months, the VPD in glasshouses can reach >2kPa (Lu *et al.*, 2015; D. Zhang *et al.*, 2018), higher than the high VPD (low humidity) treatments during this thesis. Having demonstrated the detrimental effects of high VPD on plant physiology,

and studies such as Novick *et al.* (2016) suggesting that drier air (high VPD) is a bigger stress to plants than dry soil conditions, we have highlighted the need for commercial glasshouses to take into account the effects of VPD, to effectively provide the optimal conditions for plant growth and productivity.

In closing, this thesis has not only highlighted the importance of considering humidity and subsequent VPD, when investigating the effects of soil moisture on plant physiology at early developmental stages but has also how two different species with different photosynthetic pathways and levels of isohydricity also impact such responses. Humidity can be a help or hindrance to early plant growth and productivity, depending on the soil moisture content. Furthermore, with our ever-changing climate, it is important to develop our understanding of such responses so that future crop productivity can not only be maintained in adverse conditions but improved to support our rising global population

6.4 FUTURE EXPERIMENTS

The research carried out within this thesis has highlighted interesting physiological effects of humidity and soil moisture on maize and wheat, as such there are numerous future experiments and considerations for an experimental design that would help to build upon the findings established throughout this thesis. Future experiments containing more replicates and growth in treatment conditions for a longer period would increase exposure time and possibly reduce variation in the data. It would be beneficial to grow plants to maturity to see how the treatment effects influence yield and reproduction, to aid future predictions of longer-term effects on plant physiology at different developmental stages. The

following potential future experiments would be useful to build on knowledge gained in chapters throughout the thesis.

Due to the different optimal growth conditions with regards to temperature, I would recommend that future experiments comparing maize and wheat would grow the crops separately in their optimal growth temperatures. Due to limited space and time both crops were grown in the same environments, which inevitably led to one species being closer to optimum conditions compared to the other, which may have influenced their sensitivity and subsequent response to treatment conditions. During Chapters 2, 3 and 4 growth took place in a glasshouse which reached higher temperatures which were more suited to maize, whereas in Chapter 5 during the controlled growth cabinet, lower temperatures were achieved which were more suited to wheat. Though the maize and wheat grew well in both growth environments, with no visibly obvious signs of severe stress we cannot rule out the possibility that some treatment responses were also down to how stressed or not the plant was in the growth environment. Future experiments should therefore grow plant species in their optimal growth environments to be able to determine if physiological responses are solely down to the treatment conditions.

Chapter 3 ABA concentration

It would be beneficial to measure ABA concentrations over a time-course experiment to shed light on the diurnal ABA fluxes in the plant system and how they are affected by the treatment conditions. Measuring the ABA concentration in the xylem sap will also help to build the picture of ABA movement within the plant system. In addition through also measuring the pH in both xylem sap and leaves, could help establish how ‘sensitive’ the plants are to the treatment

conditions and help to explain how in some cases high concentrations of [ABA] do not always elicit a response (e.g. stomatal closure). To further explore the effects of ABA in above and below-ground organs in response to humidity and soil moisture content, a grafting experiment with ABA mutants could be carried out. This will help to further decouple the influences of soil moisture and humidity on [ABA] within the plant system and aid our understanding of ABA movement under the humidity and soil moisture treatment conditions.

Chapter 4 root anatomy and leaf cuticle chemistry

Building upon findings from Chapter 4 it would be advantageous to conduct a time-course experiment to assess the growth rate and accurately estimate the age of tissues sampled for root anatomy analysis, as age can affect tissue formation. By conducting the experiments over a longer period, we could sample other root types such as crown roots in maize, which would shed light on how different root types response to treatment conditions. With regards to the cuticle chemistry data, whilst a longer-term experiment would be valuable to expose the plants to treatment conditions over a longer timeframe, more specific methods could help distinguish more specific chemical changes in the chemistry of the cuticle particularly waxes. Methods carried out in Razeq *et al* (2014) would be useful in the characterisation of chloroform-extractable waves, from above and below ground organs, giving more of a whole plant perspective.

Chapter 5 Whole plant physiology

To further explore the potential effects of humidity and soil moisture on plant productivity and to address some of the experimental concerns raised during this study, more work is needed. A soil compaction study including the measurements of soil penetration resistance alongside a study like this would help to assess the

likelihood of the experimental treatments (e.g. low soil moisture) and procedures (watering) causing soil compaction and increased mechanical impedance to root growth. With regards to root system architecture, a time series experiment, measuring root emergence and growth rates would further increase our understanding of how the treatments are affecting root growth. Carrying out such experiments in larger pots would enable longer plant growth and the observance of emergent root angles to help assess a plants exploration strategy before plants become pot bound. Furthermore, by increasing duration of the growing period, we increase the length of exposure to treatment conditions and may begin to observe effects, that were not previously detected in short term experiments looking at early plant growth.

Further exploration of the effects of high humidity on maize stomatal conductance and photosynthetic parameters under varying light intensities (which will be measured throughout the experiment) will help to replicate natural conditions and determine whether high humidity has as negative of an effect on the photosynthetic capacity when light levels lower, reflecting the changes in incidence radiation when cloud cover is present. Such conditions are more representative of high humidity environments whereby cloud cover usually increases with increased relative humidity.

Possible Foliar water uptake and water potential study

An experiment that could complement the findings of this thesis, would be one to test for possible foliar water uptake whilst measuring leaf water potentials, under the humidity and soil moisture treatment conditions. This further work could involve a submergence experiment into deionised water to determine whether

maize and wheat leaves can partake in foliar water uptake, with leaf water potential measurements taken before and after submergence. The experiment could follow the methods carried out in the study by Eller, Lima and Oliveira (2016). This experiment would not only explore whether foliar water uptake is occurring but also test whether plants grown in high humidity conditions, are more efficient and take up more foliar derived water when soil moisture is limiting. Through investigating how leaf water potential is affected by treatment conditions could also provide an insight into how humidity could help or hinder maintenance of leaf water potential under high and low soil moisture conditions. Furthermore, through running both the leaf water potential measurements alongside a submergence experiment, observations on how different treatment conditions affect the plants response to submergence and how effective they are at maintaining leaf water potentials and thus maintain leaf turgor.

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